Proceedings

RIAGE

of Irradiated Personnel

An
Armed Forces Radiobiology
Research Institute
Workshop

25-27 September 1996

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of Irradiated Personnel

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Preface

The workshop, "Triage of Irradiated Personnel," sponsored by the U.S. Army Office of the Surgeon General, was conducted at the Armed Forces Radiobiology Research Institute (AFRRI) on September 25–27, 1996. This workshop focused on a reassessment of the radiation medicine section of Chapter 4, *Medical Aspects of Nuclear, Biological and Chemical Warfare,* of *Army Field Manual 8-10-7: Health Service Support in a Nuclear, Biological and Chemical Environment.* Sixty-five speakers and guests from the United States, Germany, Netherlands, Canada, United Kingdom, and France addressed the three issues: (1) operational effectiveness of exposed personnel with and without other injuries who receive medical care in Army echelon I and echelon II medical facilities, (2) operational effectiveness of personnel with multiple exposures (assuming a previous total dose of <1.5 Gy), and (3) methods for estimating exposure in personnel who receive antiemetics before or shortly after exposure.

Over the course of the 3-day workshop the following four sessions were conducted:

Session I. Background. An overview of doctrine and laboratory capabilities in a forward field medical environment and an assessment of the future operational capabilities of forward-deployed medical units and supporting laboratories.

Session II. Estimation of Exposure Using Blood Markers and Clinical Indicators. Assessment of the accuracy, sensitivity, and reliability of monitoring blood-cell responses and other clinical indicators, particularly if nausea and vomiting have been reduced or eliminated by the administration of antiemetics.

Session III. Predicting the Effects of Multiple Radiation Exposures. Assessment of the accuracy, reliability, and validity of animal and human data that have been used to predict the outcome of exposure scenarios.

Session IV. Forward-Field Bioindicators for Dose Assessment: Possible Alternatives. Evaluation of the status of several possible alternatives to peripheral blood counts and prodromal symptoms for field-dose assessment. Selected candidate assays were evaluated; characteristics included (1) negligible post-sampling incubation, (2) rapid processing suitable for a high degree of automation and high throughput, (3) low-threshold and broad dose-range capability, (4) relatively noninvasive sample collection, and (5) equipment hardware for which components are or soon will be available.

At the conclusion of each session a panel of invited speakers and AFRRI subject-matter experts discussed the presentations and session findings. Audience participation generated several questions and comments. The final morning summary session addressed the issues and highlighted the workshop conclusions as well as the remaining uncertainties.

The findings of this workshop are intended for use by U.S. Army medical planners, particularly those involved in the configuration, deployment, and logistics of forward-field medical facilities (echelon I and II). These Summaries do not necessarily reflect either current or future Army doctrine. It must be emphasized that triage is a dynamic process that includes prioritization at each iteration. The initial categorization and treatment delivered to the patient will require and receive reevaluation as the patient's clinical course develops and as echelons of available care and resources change. Physicians at each echelon of care will determine appropriate management based primarily on their clinical impression of the patient's condition and what means of intervention appear best suited to favorably influence the course of disease. Laboratory studies, including physical dosimetry—no matter how accurate—are used to support clinical judgment, not substitute for it. One final point: triage is NOT a decision based on military utility to "treat or not treat." It is a decision on

how best to use both personnel and materiel resources to maximize treatment for *every* patient and to achieve favorable clinical outcomes for as many patients as possible. Although the concept of triage is generally cited in a military medical context, it is a *de facto* practice in every busy emergency room and in every major disaster, civilian or military.

The success of this workshop is directly attributable to the excellence and expertise of the participants. In addition, each session chairman deserves praise for the formidable amount of preparation and knowledge—without which this workshop could not have succeeded. COL David G. Jarrett, M.D., USA, Session I Chairman; Dr. Thomas M. Seed, Session II Chairman; Dr. Gregory L. King, Session III Chairman; and Dr. William F. Blakely, Session IV Chairman, deserve high praise for the success of this endeavor.

GLEN I. REEVES
Colonel, USAF, MC, SFS
Workshop Coordinator

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Summary of Session I

Background

David G. Jarrett, COL, MC, USA
Chairman

Armed Forces Radiobiology Research Institute, Bethesda, MD

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  Gary C. Norris, LTC, MSC, USA
  Directorate of Combat and Doctrine Development
  Army Medical Department (AMEDD) Center and School
  Fort Sam Houston, TX

- Overview of Current Operational Policy
  Robert Mosebar, COL, MC, USA (ret)
  Directorate of Combat and Doctrine Development
  AMEDD Center and School
  Fort Sam Houston, TX

- Field Laboratory Capabilities
  Samuel J.P. Livingstone, Maj, MSC, USAF
  Wilford Hall Air Force Medical Center
  Andrews Air Force Base, MD

- Status and Limitations of Physical Dosimetry in the Field Environment
  David A. Schauer, LCDR, MSC, USN
  Naval Dosimetry Center
  Navy Environmental Health Center Detachment
  Bethesda, MD

- NATO Policy and Guidance on Antiemetic Usage
  Robert Kehlet
  Defense Special Weapons Agency
  Alexandria, VA

- Review of Potential Biomarkers of Radiation Exposure
  Dr. Clive L. Greenstock
  Radiation Protection Branch
  Atomic Energy Commission Limited, Chalk River Laboratories
  Chalk River, Ontario, Canada

Overview

The goal of Session I was to provide background information and a starting point for the many physicians and researchers who are not acquainted with the capabilities and limitations of providing medical care away from a permanent medical facility. The current and near-future deployable medical units were discussed and an overview of potential radiation biomarkers was presented.

Introduction

The United States Army must be prepared to effectively use soldiers in a radiologically contaminated environment during small-scale operations that include accidents and terrorist activity and in the event of a nuclear conflict. Current doctrine is based on scenarios of cold war transition to nuclear war and the resultant mass-casualty medical requirements. Early return to combat duty would be practiced for all soldiers who are nominally performance capable. Significant morbidity and mortality of radiation casualties would not occur until after the arrival of reinforcements. Isolated radiation injury does not necessarily cause significant immediate disability; and truly effective therapy for radiologically injured soldiers is considered improbable in a total nuclear war scenario. However, low- to mid-range radiation injury presents a unique problem as acute symptoms can be debilitating but are preventable with adequate prophylaxis. If left untreated, the hematologic intermediate-term (i.e., 2 to 6 weeks) effects of this same radiation injury would become devastating.

In view of the significant advances in the treatment of hematologic injury as well as changes in military operational requirements, a review of current doctrine is mandatory. The purpose of this section was to present to scientists the medical capabilities of deployable units to ensure that recommendations for implementing triage mechanisms will be practical.

Military Medical Care at Far-Forward Echelons

Military medical care is designed to be delivered as far forward as is practical. This doctrine maximizes the return to duty of individuals with minor injuries and makes a positive impact on battle outcome. The early evacuation of casualties requiring prolonged treatment concomitantly minimizes the individual casualty's morbidity and frees forward-medical resources to concentrate on short-term care. No current method is available to rapidly assess an individual's degree of radiologic injury, and as radiation is not likely to result in immediate mortality, triage is primarily based on other criteria. Those soldiers in whom radiation injury is suspected would require evacuation to the hospital level for evaluation.

Initial treatment may be provided by the combat life-saver, a combat soldier trained in advanced first aid techniques. The first medical treatment (echelon I) is provided by the combat medic and his supervising battalion aid station (BAS), which has a physician and a physician's assistant. No laboratory or radiologic equipment is available at the BAS. The two basic choices are either treatment to allow return of the casualty to duty or stabilization for evacuation to echelon II or III. At echelon II (the medical company), rudimentary laboratory services and x-ray capability are available as are holding beds for patients who are expected to return to duty within a well-defined short time frame. Interventional surgery can be placed at this level as an augmentation module when additional forward capability is deemed practical. Echelon III is the first hospital facility and has the capabilities for true blood-cell counting and limited chemistry. Most patients who are evacuated to this level will proceed up the evacuation chain and will not return to duty soon. The next echelon of evacuation will be to a theater-level medical facility, a fixed-base facility, or a continental U.S. (CONUS) hospital. See Fig. 1 for possible routes of medical evacuation from the far-forward field.

The Theater Army Medical Laboratory (TAML) is an independent field laboratory capable of providing regional support that includes clinical laboratory reference testing for biochemical, toxicological, bacteriological, mycological, and parasitological agents. It is also capable of gross and microscopic pathology support. Its medical defense tactical applications include confirmation of endemic disease and suspected radiological, chemical, and biological warfare agents. Future plans include replacement of the TAML with an Area Medical Laboratory (AML),

which is smaller and requires less logistical support (diminished footprint); the rapid diagnostic and chemistry test capabilities will be relocated to echelon II medical companies. The AML will then be focused directly on battlefield health-hazard assessment.

Current Operational Policy

Symptoms of radiation injury will usually not be manifest at acute doses of less than 100 cGy but will be progressively more intense and more rapid in onset with higher radiation doses. Most soldiers who receive low to midrange radiation doses (100–300 cGy) will have symptoms of nausea and vomiting within several hours of exposure and are consequently less tactically effective. They are then significantly more prone to further traumatic injury as they can less proficiently operate weapon systems,

defend themselves, and press the attack. Primary clinical guidance for triage of radiation casualties with unknown dose is based on symptoms during the prodromal period. This is the interval between time of exposure and cessation of nausea and vomiting. Radiation exposure dose is estimated based on the time interval between exposure, symptom onset, and symptom severity. To diminish the individual soldier's morbidity and performance degradation, the development of a safe prophylactic drug for the nausea and emesis of significant radiation injury was necessary. Use of this medication would prevent individual capability degradation and would consequently diminish the overall casualty rate by allowing tactical mission completion. Unfortunately, eliminating these symptoms rules out the medical officer's ability to clinically estimate radiation exposure without research-level diagnostic modalities.
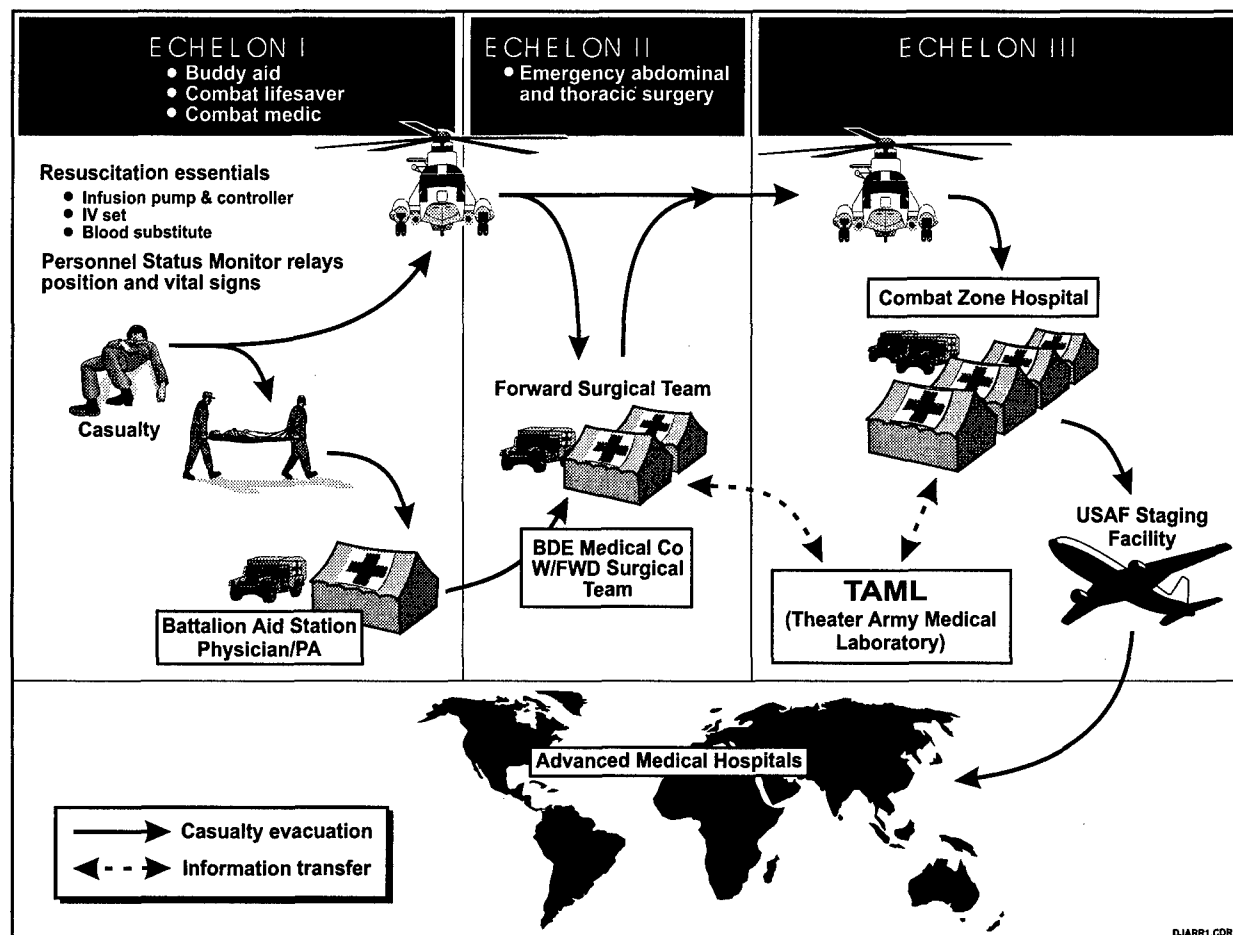

Fig. 1. Routes of medical evacuation from the far-forward field.

Status and Limitations of Physical Dosimetry in the Field Environment

Peacetime occupational radiation exposures are monitored using thermoluminescent dosimeter (TLD) systems accredited by the National Voluntary Laboratory Accreditation Program (NVLAP). These systems are designed for centrally located and controlled programs and are not suitable for field operations. War-time dosimetry is based and controlled at the unit level. The primary individual dosimeter system currently fielded is the high-range photoluminescent AN/PDR-75. This system consists of the ruggedized DT-236 wristband dosimeter that is capable of measuring cumulative neutron and gamma-ray doses in the range of 0–999 cGy and the CP-696 nondestructive dosimeter reader. The CP-696 is issued to company-sized units, and results are recorded at company level. The system is not considered a medical-issue item, and dosimeters are not routinely distributed to deploying soldiers. Future dosimetry systems include the platoon-based AN/UDR-13, a direct-reading dose-rate meter and a total gamma-plus-neutron dosimeter. Next-generation systems should include the capability of teledosimetry that allows remote monitoring of individual dose-rate exposure.

Current NATO Policy and Guidance on Antiemetic Usage

The North Atlantic Treaty Organization (NATO) project group (PG-29) has recommended granisetron as the deployable radiation emesis prevention drug of choice. NATO members will develop individual operational plans for implementation under the draft Standardization Agreement (STANAG 4510). Under draft STANAG 4511, multiple prophylactic antiemetic medications and regimens were evaluated prior to adoption of granisetron.

Two drugs exceeded the criteria (shown below), granisetron and ondansetron. The former was adopted due to a better technical profile and the operational advantage of once daily oral administration.

Review of Potential Biomarkers of Radiation Exposure

The human body is the ultimate dosimeter, and measurable changes in tissues and biosamples are the most important indicators of whole-body dose. Two types of biodosimeters are possible: biophysical indicators that are direct measures of absorbed

Summarized NATO criteria for an acceptable antiemetic

Approval by individual national pharmaceutical regulatory authorities.

Effective antiemetic for radiation doses up to 10 Gy.

Rapidly effective after a single self-administered oral or auto-injectable dose.

Compatible with other normal prophylactic and emergency therapeutic measures.

No militarily significant side effects, and no potential for abuse.

Effective in extremes of climate without compromising individual environmental conditioning.

Packaged for 1-week dosages utilizable by individuals in protective equipment.

Minimum of 36-month shelf life stability between 0 and 50 degrees Celsius.

dose and are not influenced by repair mechanisms, and biological indicators that record absorbed dose by a biological manifestation of radiation exposure. A biophysical indicator, such as electron spin resonance (ESR), is instrument-based and is usually more amenable to automation. It is analogous to a physical personal dosimeter. A biological dosimeter can include physiological, hematological, bio-chemical, immunological, and cytological assays. Consequently, it measures the biologically relevant effects of radiation to estimate the effective dose received. Testing usually requires experienced technicians to minimize method variability and subjectivity for reliable dose estimation. Relevant portions of this talk are summarized in subsequent sessions.

Summary of Session II

Estimation of Exposure Using Blood Markers and Clinical Indicators

Thomas M. Seed, Ph.D. [1]
Chairman

CDR Michael E. Dobson, LTC Daniel C. Garner,
LT Tracy B. Kneisler, and Dr. Mark H. Whitnall
Session Panelists

Armed Forces Radiobiology Research Institute, Bethesda, MD

- Alterations of Hematological Parameters by Radiation
  Niel Wald, M.D.
  Department of Environmental and Occupational Health, Graduate School of
  Public Health
  University of Pittsburgh, Pittsburgh, PA

- Prediction of Clinical Course Through Serial Determinations
  Hauke Kindler, D. Densow, T. M. Fliedner
  Institute of Occupational and Social Medicine, University of Ulm, Ulm, Germany

- Dose Estimation Using Lymphocyte Depletion Kinetics
  Ronald E. Goans, Elizabeth C. Holloway, Mary Ellen Berger, Robert C. Ricks
  Radiation Emergency Assistance Center/Training Center (REAC/TS)
  Oak Ridge Institute for Science and Education
  Oak Ridge, TN

- Fatigability and Weakness as Clinical Indicators of Exposure
  George H. Anno
  Pacific-Sierra Research Corporation
  Santa Monica, CA

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Overview

This session's goal was to examine the usefulness and practicality of attempting to assess—for the purpose of triage—early clinical and blood-cell responses that would occur in irradiated military personnel operating within radiation contaminated far-forward battlefields.[1]

The operational necessity to rapidly and accurately assess the physiological responses induced by radiation exposure and the possible concomitant negative impact on troop performance (and to a lesser extent, long-term medical complications and subsequent treatment protocols), provided the orientation for this session's presentations and topics.

The session featured four presentations by invited experts who spoke on various aspects of radiation-induced blood alterations, the clinical responses, and their applicability as clinical indicators for medical triage of irradiated military personnel in the far-forward field.[2] Two presentations (by Drs. Niel Wald, University of Pittsburgh, and Hauke Kindler, University of Ulm) provided overviews of the temporal and exposure-dependent hematological response patterns of acutely irradiated individuals. An additional presentation (by Dr. Ron Goans, REACT/S, Oak Ridge) focused on the biodosimetric potential of assessing lymphocyte depletion kinetics. The fourth presentation (by George Anno, Pacific-Sierra Research Corp.) discussed the possibility of using the clinical responses of fatigability and weakness as early indicators of the extent of radiation exposure.

An open discussion followed the presentations in which presenters and audience alike examined and provided comment, not only on the accuracy, sensitivity, and reliability of these measured, well-documented clinical and hematological endpoints, but also on the practicality of such assessments given the constraints of a narrow time window (24-hr postexposure), the possibility that antiemetics were given, and the far-forward medical echelon I and II settings.

The general consensus was that under such constraints (time, technology, and echelon setting) these blood and clinical indicators are not adequate for initial triage in the far-forward field. First-line triage would be better served by the application of physical dosimetry, rather than by blood/clinical indicator-based biodosimetric procedures.[3] The latter procedures would serve a more useful function in secondary triage processes during which clinical management/treatment decisions can be made.

Presentation Details and Comments

Comparisons of attributes of the three blood marker-based assays, along with a single clinical-indicator assay, are presented in Table 1.

Blood Markers in Estimating Exposure

A single parameter assessment strategy, offered by Dr. Ron Goans (ORISE, Oak Ridge, TN), is based on monitoring the rate of lymphocyte depletion during the initial 24-hr postexposure period. This biodosimetric tool seems to provide a moderately

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[1]Triage processes discussed relate solely to military operations under battlefield conditions, and not to possible early processing of civilian casualties by civilian medical doctors following a nuclear accident.

[2]An additional presentation relevant to this topic of early hematopoietic response indicators was made by Dr. L.G. Filion (University of Ottawa, Canada) during Session IV. For details of this presentation, the reader should refer to Appendix D, which contains the appended text of this work.

[3]Proposed use of physical dosimetry in forward fields of operation is not intended to supplant the need for medical evaluation by either the medic in the field or by the physician in a higher echelon care facility, but only as a means to more effectively sort minimally exposed "duty-ready" troops from those troops deemed "suspect" and perhaps "duty unfit" due to moderate-to-heavy exposures and associated performance and health status degradations.

reliable and consistent but fairly crude assessment of acutely delivered whole-body exposures.[4] It has the strongest biodosimetric potential in the far-forward field of operation of various assays described during this session. The technique, however, is limited in terms of threshold doses to approximately 1 Gy and to approximately 2–3 Gy in terms of resolving distinct exposure levels within the range of detection, i.e., ~1–10[+] Gy. The relatively short time (6–8 hrs) for assay development is a major advantage; whereas the requirement for multiple sampling and the uncertainty of confounding factors (biological warfare (BW)/chemical warfare (CW) agent exposures, combined wound/burn injuries, physiological stress, etc.) represent major disadvantages.

This assay technique should be considered only in terms of its dosimetric attributes, and not as a diagnostic tool upon which treatment decisions are based.

Two **multiparameter strategies** for the hematologic assessment of the "acute radiation syndrome (ARS)" were presented—one by Dr. Niel Wald of the University of Pittsburgh, and another by Dr. Hauke Kindler of the University of Ulm (Ulm, Germany). Both strategies were designed to segregate radiation-exposed individuals into distinct severity levels (five levels) of radiation injury (ranging from minimal, nonlethal, to severe, definitively lethal), thus, providing the clinical rationale for subsequent treatment options.

Dosimetric assessment was not the primary objective of these assessments per se; although crude estimates of exposure levels can indeed be determined based on characteristic blood response profiles.[5]

The approach outlined by Wald involves ranked analyses of the degree of exposure-dependent

Table 1. Comparison of attributes of hematological/clinical indicator-based assays.

Attributes	Assay types			
	Hematologic-based			Clinical-based
No. endpoints	1-parameter	3-parameter	9-parameter	F/W
No. samples required	≥3	≥6	≥6?	>1?
Predictor function	weak	strong	strong	very weak
Biodosimeter function	moderate	weak	weak	negligible
Threshold dose	~1Gy	~1Gy	~1Gy	~<1Gy
Resolution	~2Gy	~2–3Gy	~2–3Gy	~2–3Gy?
Range of detection	~1–10+Gy	~1–10+Gy	~1–10+Gy	~1–10+Gy?
Validation of test	yes	yes	yes	?
Development time	≤24hrs	1–5d	4–7d	≤24hrs
Confounders	yes	yes	yes	yes
influence	+	-	-	+
-stress	+	±	±	±
-combined injury -BW/CW	+	±	±	+
Meets far forward- field use requirements	no	no	no	no

[4]The utility of such "lymphocyte" depletion-type assays to accurately and reliably estimate radiation exposure levels has been actively debated and questioned, especially within the community of physicians and researchers specializing in radiation hematology.

[5]Clinical assessments based on blood-cell counts should be used first and foremost to estimate both the extent of hematopoietic injury, as well as the capacity for injury repair, and should provide the basis for rational, medically-based, treatment decisions. Extracting prognostically useful information from simple blood-cell responses is quite possible but is clearly and absolutely time dependent.

hematologic abnormality. The assay is based on the serial assessment and abnormality scoring of nine individual blood parameters (hemoglobin levels, erythrocyte counts, hematocrit values, erythrocyte sedimentation rates, reticulocyte counts, total leukocyte counts, absolute neutrophil levels, lymphocyte counts, and platelet levels) and, in turn, the folding of the individual abnormality scores into a single score of hematopoietic injury.

The major advantage of this approach is its strong predicator function of clinical outcome; whereas its major disadvantages are the relatively long development times (~1–5 days) and low resolution of the dose-dependent clinical responses.

The ARS assessment approach presented by Kindler is similar to Wald's approach, in terms of its multi-parameter nature, but employs fewer parameters which are analyzed individually and not in aggregate at distinct times over the course of the initial week following exposure. This assessment process has been labeled the "sequential diagnosis" technique.

Response profiles for given parameters were based on a detailed, retrospective study of 543 ARS patient records from the International Computer Database for Radiation Exposure Case Histories (ICDREC). These profiles were used in determining patient management/treatment decisions. It should be noted that the majority of cases (390/543 or 71%) exhibited minimal injury profiles and required no specific treatment for radiation injury. The next largest group (101/543 or 18%) had substantial levels of injury but were clearly treatable with standard clinical support and cytokine therapy. Only a minor group (52/543 or 9%) exhibited radiation injuries so severe as to require intense, technologically complex levels of support (e.g., marrow transplantation).

Based on data presented by Kindler, reliable assessments of injury severity can be made within a 4- to 7-day window if blood granulocyte, lymphocyte, and platelet levels are sequentially monitored (at 6-hr intervals during the initial 36-hr postexposure period). However, accurate dose-dependent injury assessment within the targeted 24-hr postexposure period is not possible using this assay procedure.

The multiparameter assessment (sequential diagnosis) procedure developed by the Ulm group (led by Professor T.M. Fliedner) has strong prognostic capabilities and can provide a solid basis for subsequent medical management and treatment decisions. When compared to Wald's "folded multiparameter" scoring assay, this procedure enjoys roughly the same sensitivity (~1 Gy), resolution (~2–3 Gy), as well as the overall range of detection (~1–10+ Gy). The major weaknesses (in terms of its biodosimetric potential) are again the long development times (~4 to 7 days) and the requirement for multiple sampling.

Clinical Indicators in Estimating Exposure

A physiological-based model using empirical observations of the well recognized radiation associated clinical syndrome of fatigability and weakness (F/W) were presented by Mr. George Anno of Pacific-Sierra Research Corporation. This syndrome was discussed, not only in terms of eliciting radiological and temporal parameters, but also as a very early (<24 hrs) indicator of the magnitude and occurrence of radiation exposure.

This discussion was driven by underlying questions as to whether F/W can serve as a reliable, meaningful indicator of radiation exposure if the prominent clinical indicators of nausea and vomiting are stripped away by the application of antiemetics. The consensus answer to this question was clearly and simply—no.

The F/W model, founded on a lymphocyte-depletion kinetics construct, seems to provide reasonably consistent exposure-dependent response profiles (comparing "observed" to "expected" response profiles). This consistency along with early assessment times of 24 hrs or less and the prospect of indirectly quantifying the highly subjective F/W parameter through a simple lymphocyte count are all attractive attributes. Nevertheless, near-term application of the F/W modeled parameter to triage in the forward field is extremely doubtful, due not only to the uncertainty of individual response variations (lack of confidence intervals) but also to the uncertainty of the basic mechanism(s) governing the F/W response.

Discussion and Conclusions

This session, with its focus on "blood markers and clinical indicators," primarily addressed the third issue raised in Chapter 4 of the *Army Field Manual 8-10-7: Methods for Estimating Exposure in Personnel Who Have Received Antiemetics Before or Shortly After Exposure.*

In principle, both the single- and multiparameter-hematologic methods described during this session can be used to provide estimates of exposure within irradiated personnel regardless of whether antiemetics have been given.

The sensitivity, resolution, and range of detection displayed by these assays are reasonable and probably would be effective as supplemental procedures for triage under select conditions. For example, the rapid one-parameter/lymphocyte-depletion kinetics assay might be suitable to provide rough estimates of radiation casualty numbers needed for medical logistics; whereas the slower, more prognostic multiparameter assays (3- and 9-parameter assays) might be effectively applied for determining the extent of injury and subsequent treatment options. However, the utility of these procedures for triage in a forward-field operation is questionable. This concern was raised principally by Dr. Robert H. Mosebar, a recently retired chief medical operations planner (U.S. Army Medical Department Center, Fort Sam Houston, Texas) and by others in the audience as well. The constraints of time, medical resources, and the operation itself seem to preclude use of these assays. The status of existing blood-analysis technology restricts these assays to a minimum echelon II facility—a facility designed for high throughput of short-term emergencies, and not for the prolonged sequential monitoring required by

these hematologic assays, especially the multiparameter-based assays.[6]

Nevertheless, the multiparameter hematologic assays and their strong prognostic attributes are absolutely essential, perhaps not in the very early phases of triage but a little downstream in the process. These assays are essential in determining the severity of radiation-induced injuries and the probability of organ-system repair and recovery. Such determinations provide the basis for subsequent treatment decisions.

In contrast to the hematologic assays, it is extremely doubtful that the clinical F/W response assay, as it currently stands, can provide a useful vehicle for estimating exposure levels, regardless of whether antiemetics are administered. The highly subjective nature of the F/W clinical response and the lack of quantitative methods for determining its measurement, effectively rules out the use of this endpoint as a biodosimeter.

The aforementioned operational problems of using the F/W bioassay in the initial triage of radiation-injured personnel in far-forward combat environments led to considering physical dosimetry as a possible interim solution to the rapid (<24 hrs) throughput requirements for the initial phase of the triage process.[7] Dosimetry could be rapidly read, exposure levels estimated, and casualty probabilities determined—all in an echelon II facility. With these dose estimates, troops with registered doses (free-in-air) of 1.5 Gy or greater, would be medically evacuated to an echelon III facility for clinical and hematologic evaluations on which subsequent treatment decisions would be based. Troops with estimated exposures of less than 1.5 Gy (as assessed by initial physical dosimetry) or lacking subsequent

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[6]The suggestion was offered that with the advent of new technology (in the form of hand-held blood cell analysis units) the above mentioned shortcomings of the hematologic assays in far-forward fields of operation might be largely overcome. With such hand-held units, medics should be able to perform these hematologic assays on putatively injured troops directly in the field (or in an echelon I facility) with high efficiency and accuracy. However, it needs to be noted that the utility of applying such advanced monitoring devices in far-forward fields of operation has been seriously questioned by several radiation hematologists.

[7]The suggested use of physical dosimetry in the initial phases of the triage process is intended to supplement, not to minimize, nor eliminate the need for full symptomatologic assessment by the field medic or physician in a forward-field care facility. Physical dosimetry is suggested as a possible solution to the substantial problem imposed by the constraints of time, resources, and casualty-care logistics under battlefield conditions. See footnotes 1, 3, and 5 for additional comments.

clinical/hematologic indicators of radiation injury (assessed in the echelon III facility) would be sent back to duty.

The remaining two issues addressed by the workshop, namely, (1) operational effectiveness of irradiated personnel, and (2) operational effectiveness of previously exposed personnel, were only briefly considered, specifically in terms of the expected performance decrement following onset of selected clinical syndromes (upper/lower gastrointestinal and hematologic syndromes). Changes in operational performance caused by radiation exposure need to be considered within a temporal framework of both "early effects" (less than 24 hrs) and "late effects" (>24 hrs, days to weeks). In terms of the latter, the consensus was clear: The prognostically useful hematologic assays relate to long-term performance and not to short-term capacity, due to the delayed onset (days to weeks) of the potential, performance-degrading hemopathologic responses (granulocytopenia and thrombocytopenia, with increased susceptibility to infection and uncontrolled bleeding).

The dose-dependency of decreased performance under acute radiation exposure scenarios has been extensively studied and modeled (as indicated in the Session III presentation by Dr. Gene McClellan of Pacific-Sierra Research Corp.). However, the appropriateness of these models, either in terms of their basic biology or to the triage process itself, was not fully addressed.

Early changes in operational performance can be elicited by induction of the F/W syndrome. Even at fairly moderate radiation doses, where long-term survival would be expected, there is rapid onset (<~3 hrs) of a moderately intense F/W response with the potential to degrade short-term performance (<24 hrs). In this regard, there was general agreement among workshop participants that (1) the F/W syndrome is a very real and important component of performance and (2) its induction and expression is governed by a standard set of radiological parameters. However, there was also agreement that the F/W response is highly subjective and resists quantitation.

This restricts the assignment of confidence intervals to F/W response components (threshold, magnitude, intensity) and, in turn, limits its power and utility as a "folded parameter" in the model of radiation-associated performance degradation.

Recommendations

1. Physical dosimetry should be the principal tool in the initial assessment of radiation exposure levels received by military personnel operating within far-forward fields of operation.[7]

    1.1. Exposure estimates would be carried out in an echelon II facility and would be used solely for logistical purposes and not for treatment decisions.

    1.2. Personnel with estimated exposures of 1.5 cGy or greater (free-in-air doses) would be medically evacuated to an echelon III facility for confirmation of performance/health degrading radiation exposure. Personnel with estimated exposures of less than 1.5 cGy would be deemed fit and would be returned to duty.

2. Application of the slower developing, highly prognostic, multiparameter hematologic assays should be restricted to echelon III facilities. These assays would be applied to (a) confirm initial exposure assessments made by physical dosimetry in far-forward fields of operation, (b) assess the extent of radiation injury to the vital lymphohematopoietic system and, (c) provide the basis for subsequent treatment decisions.

3. The possibility of far-forward fielding the single parameter lymphocyte-depletion assay for biodosimetry needs to be reconsidered and delayed until (a) its efficacy is more thoroughly evaluated, (b) confounding factors affecting assay performance are more fully determined, and (c) hardened, hand-held electronic blood cell counting devices become available.

3.1. Comprehensive testing of the assay and the instrument will be required prior to any consideration of fielding.

4. F/W syndrome should not be considered as an adequate substitute for the more demonstrative clinical indicators (nausea and vomiting) of radiation exposure, regardless of whether anti-emetics are involved.

4.1. Improved methods to better quantitate F/W are needed to support radiation-associated performance decrement evaluation models.

Summary of Session III

Predicting the Effects of Multiple Radiation Exposures

Gregory L. King, Ph.D.
Chairman

André Dubois, M.D., Ph.D., LTJG Matthew Hamilton, MSC, USN,
Michael R. Landauer, Ph.D., and G. David Ledney, Ph.D.
Session Panelists

Armed Forces Radiobiology Research Institute, Bethesda, MD

- Large Animal Radiation Experiments
    E. John Ainsworth, Ph.D.
    Armed Forces Radiobiology Research Institute
    Bethesda, MD

- Estimating Lethality Risks for Complex Exposure Patterns
    Bobby R. Scott, Ph.D.
    Lovelace Research Foundation
    Albuquerque, NM

- An Integrated, Physiologically-Based Model of Human Response to Multiple
Exposures
    Gene McClellan, Ph.D.
    Pacific-Sierra Research Corporation
    Arlington, VA

- Operational Performance Decrement After Radiation Exposure
    George H. Anno
    Pacific-Sierra Research Corporation
    Santa Monica, CA

Overview

This session's goal was to describe the impact on the operational effectiveness of military personnel who have received multiple radiation exposures within a relatively short time frame and who have not been medicated. Participants were asked to estimate the effects of two low-dose radiation exposures—without medication—on the human response by either mathematical models or extrapolation from published human and animal data. While the mathematical models provided useful general predictions, most of the workshop participants agreed that further validation of these models was necessary. The panelists and participants also agreed that no animal model completely predicts the effects of acute, protracted, or multiple radiation exposures in man; and that fatigability and weakness would worsen with multiple radiation exposures regardless of the fractionation interval between exposures. An effort was made to incorporate the data and models that were presented into a table for use in the *U.S. Army Field Manual 8-10-7, Health Service Support in a Nuclear, Biological, and Chemical Environment.*

Introduction

The goal of this session was to describe the injuries of military personnel who have been exposed to multiple radiations within a relatively short time frame and who do not receive medication, as well as to describe the impact of these radiation injuries on operational effectiveness. Due to the paucity of human data that directly bear on this issue, the session focused on assessing the accuracy, reliability, and validity of both animal and human data that have been used to predict outcomes from such scenarios. Because of the infinite possibilities of scenarios for multiple-radiation exposures, the participants were requested to limit their presentations and analyses to two specific time-related scenarios that had varied radiation doses. Both scenarios were for two radiation exposures separated by 7 days. The first exposure was a midline-total dose (MTD) of 0.7 Gy, given promptly (Scenario 1) versus a protracted dose over the course of 7 days (Scenario 2). The second exposure for both scenarios was a variable prompt dose: 0.5, 1.5, or 3.0 Gy. In general, military personnel exposed to a dose

of 1.5 Gy or greater will not be allowed exposure to a second radiation incident. Thus, the lower 0.7-Gy radiation dose was chosen for the first exposure.

Large Animal Radiation Experiments

This presentation summarized numerous fractionated-radiation experiments that were performed prior to 1975 on sheep, swine, goats, and dogs at the U.S. Naval Shipyard in San Francisco, California, and on non-human primates at the School of Aviation Medicine in San Antonio, Texas. All of the experiments used two radiation exposures of varying doses and were designed to determine animal recovery or "residual injury" from the first radiation dose. The endpoints in most experiments were $LD_{50/30}$ or $LD_{50/60}$ survival data; and the early indicators for radiation exposure had to be inferred from the LD_{50} data, personal experience, and from examining the differences among species. In general, the first radiation dose was two-thirds of the $LD_{50/30}$ or $LD_{50/60}$ value for a given animal species and was delivered at different dose rates for many of the experiments. The second and different radiation dose was delivered at varying intervals. This general experimental design did not allow for extrapolation to the lower, more survivable doses suggested for the scenarios described for this workshop. The overall conclusions were that various species show quite varied $LD_{50/30}$ values in response to an acute dose of ionizing radiation. Furthermore, the recovery of each species from the first radiation dose, as measured by survival to a second radiation dose, also varies across species. It was emphasized that the residual injury observed in some species in response to the second radiation dose could not be predicted from hematological measures. The discussion centered on the issue of which radiation response in a particular animal species might best correlate with the human response. The general consensus was that no single species is an ideal correlate.

Estimating Lethality Risks for Complex Exposure Patterns

This presentation described a mathematical model that could predict the hematopoietic lethality for humans exposed to complex dose-rate patterns of

gamma rays. An analysis of the model, background, and definitions were also presented. The model is dose-rate dependent and was developed from data accrued from both laboratory animals and humans. In the context of this workshop, the major limitation of the model is that it can estimate lethality only if there is either no recovery or full recovery from the initial irradiation. Thus, predictions for survival cannot be accurately made if partial recovery or residual injury to the first radiation exposure exists. Again, since lethality is an endpoint of this model, the early indicators of radiation injury in humans had to be inferred. The probability of lethality is the indicator of performance decrement. Despite this limitation, the model can provide useful predictions for estimating the upper and lower limits of radiation exposure under the multiple radiation scenarios described in the workshop.

With the limitations of either no or full recovery, the results calculated for the two scenarios showed that there should be no risk of hematopoietic deaths in either scenario, provided the second radiation dose did not exceed 0.5 Gy. If the second radiation dose exceeded 0.5 Gy, the risk for hematopoietic death increased; but importantly, the lethality risk was exclusively due to the *second radiation dose*. In a brief comparison across species, the model showed that the ordinal value estimated for the radiosensitivity of humans to a prompt radiation dose falls between that of mice and dogs—which in turn are similar to goats and swine. The model also showed that the value estimated for the relative recovery capacity in humans falls near those values for sheep and goats. As emphasized in the previous presentation, data showing the necessary survivable endpoints are unavailable for the non-human primate. To its credit, the model does allow for the inclusion of protection and susceptibility factors such as wounds, burns, and medical support.

An Integrated Physiologically Based Model of Human Response to Multiple Exposures

This presentation provided an overview of the Radiation-Induced Performance Decrement (RIPD) software program that describes certain radiation effects in humans—thus calculating the severity of illness and the residual performance capability for numerous radiation scenarios. This mathematical and computer model uses several differential equations to describe and predict radiation effects under various conditions. Each sign and symptom of the model is based on changes in biological endpoints that occur in response to radiation, such as the clearing of humoral toxins, the cellular kinetics of the intestinal mucosa, and the kinetics of lymphocytes, cytokines, and bone-marrow cells. One limitation of the model appears to be that three of the symptom categories (nausea and vomiting, diarrhea, and fatigability and weakness) and the mortality incidence are based on independent kinetic models, while the other symptom categories (fluid loss, infection and bleeding, and hypotension) are slaved to these other kinetic models. Time restrictions prevented further explanation of the biological validity of this interdependence among the symptom categories. Although some of the kinetic models were derived from animal studies, and others from human data, the output from the animal data in the RIPD model were adjusted to match the human response. Another limitation of the RIPD model is that its dose-rate dependence under some conditions appears to be an extrapolation of the acute response to a prompt irradiation that is then protracted over time. Time did not allow for presentation of specific examples of these conditions. Without further clarification, it was thus unclear which specific endpoints measured in the RIPD model reflected results from specific radiation dose-rate studies, and which endpoints were extrapolated from the results seen after a prompt radiation dose. It was noted that some data for the prodromal aspects of the model were obtained from expert opinion and from questionnaires given to military personnel. The questionnaires were designed to establish correlations between symptoms (including their severities) and task performance, not between radiation dose and performance. While such input may be important to developing and establishing the model, this approach adds a degree of subjectivity to the model. To the credit of the RIPD model, some aspects of these prodromal responses have been validated in human studies on sea-sickness.

Two other important points were made during the general discussion of this model. First, the RIPD

model cannot provide an accurate estimate of the threshold radiation dose for some of the early signs/symptoms of radiation exposure. Second, it was noted that under fractionated radiation conditions, fatigability and weakness did not show a sparing effect to that fractionation. One attendee remarked and cited some older clinical literature describing results from fractionated radiation exposures that might provide useful data to incorporate into the model. While well received, the overall impression by the attendees was that this model requires further validation with other animal models, especially for multiple-dose scenarios.

Operational Performance Decrement After Radiation Exposure

This presentation described in detail the "operational performance" defined for the RIPD model in a military setting and further described how the severities of the various manifestations of radiation sickness alter performance. Numerous illustrations showed how performance levels with 95% confidence limits for specific tasks (e.g., for a member of a tank gun crew vs. a tank commander) varied with dose and time after exposure. Due to time constraints, this presentation did not provide details on the multiple-radiation scenarios that were suggested in advance. However, and of perhaps greater importance, operational effectiveness was evaluated in response to the presence or absence of an antiemetic. Since the human response to antiemetics varies considerably, and a complete set of human data under such conditions is unavailable, the speaker was asked to assume 100% efficacy of the antiemetic drugs to give the best-case scenario. The data showed, depending on the task, that nausea and vomiting were the primary causes of performance degradation; and that operational effectiveness could be improved by an antiemetic. However, the previous presentation showed that fatigability and weakness did not show a sparing effect to multiple radiation doses. Thus, some clarification is necessary to determine the degree to which fatigability and weakness vs. nausea and vomiting contribute to performance degradation after multiple exposures to radiation.

Discussion

The discussion centered on issues involved in the construction of Table 2, *Effects of a Second Radiation Dose on Combat Effectiveness of Military Personnel*, that could hypothetically be used in the Army field manual. As seen in the table, the first radiation doses in a multiple radiation scenario are placed in two bands (1–70 cGy and 71–140 cGy) and are identical to those radiation dose bands currently described in *Army Field Manuel 8-10-7*. By consensus, it was agreed that if this first dose was ≤ 70 cGy (either prompt or protracted), military personnel would remain combat effective as long as the sum of it and the second dose did not exceed 150 cGy. This should be true for short intervals of up to 14 days. Beyond 14 days, the panelists and audience could not determine whether personnel could be exposed to a greater second dose. That is, even at low radiation doses, it is unclear whether the biological repair mechanisms would render a human the same as if he or she had never been irradiated. Regardless, lymphocyte counts should be followed in such individuals. Again by consensus, the participants agreed that if the first dose were protracted and > 70cGy, any prompt second dose at any time interval after the first dose would likely evoke partial to full performance decrement in military personnel. This reflects one of the major points gained from this portion of the workshop: the symptoms of fatigability and weakness do not show a sparing effect after fractionated radiation. The workshop participants agreed that the authors of the Army field manual should provide, if possible, military medical personnel with a definition, operational or otherwise, of radiation-induced fatigability and weakness so that medics can distinguish this symptom from battle fatigue. From the aforementioned table, it also can be discerned that there are not enough data to estimate what level-III clinical remarks might be most salient for such irradiated personnel. However, under such multiple-dose scenarios; it is possible that some of the serial measures of biological dosimetry explored in other portions of the workshop could provide invaluable assistance to the field medic.

It became readily apparent from the presentations in this session that no single animal species is the best

model for the radiation response in humans. Moreover, very little data exist that describe the survival of the non-human primate after irradiation, especially in response to multiple exposures to radiation. Since the non-human primate may be the best animal model to substitute for humans, there should be further studies with them using fractionated radiation scenarios.

As a side point, it was suggested that the Armed Forces Radiobiology Research Institute should become a central repository for older government data that deal with radiation studies and should also include newer collections of human radiotherapy data. This would provide a central database that could be accessed by future generations of radiation biologists.

The mathematical models presented were sophisticated and summarized most of the existing information on human radiation effects. They are useful as working hypotheses for future testing. Unfortunately, much of the human data used to derive the models are from events in which uncertainty exists concerning radiation dose, dose distribution, and even the clinical parameters. The panelists and other participants did not have adequate time to evaluate their validity for the overall human response to radiation. The chairman and panelists of this session believe that scientific peer reviews of the models

would benefit the authors and developers of these models. These models need further work before conclusions can be derived for establishing military standards for triage. Such work should focus on validating the models across several species, with special emphasis on validating the effects of multiple radiation exposures.

Recommendations and Research Directions

The panelists and participants in this session recommended that (1) Table 2, *Effects of a Second Radiation Dose on Combat Effectiveness of Military Personnel*, should assume that no confounding variables exist (e.g., combined injury, infection, exposure to a threat agent); (2) physical dosimeters should be issued to personnel at risk of a second exposure; (3) field commanders should be made aware that the fatigability and weakness response does not show recovery; (4) multiple-radiation exposure studies should focus on end points other than lethality; (5) radiation studies should address the effects of other combined injuries, such as physical trauma or infection; and (6) an attempt should be made to quantitate fatigability and weakness in either humans or in an animal model to approximate the human response.

Table 2. Effects of a second radiation dose on combat effectiveness of military personnel.

1st dose		Prompt 2nd dose			
Prompt or protracted[1]	Time interval between doses	Maximum 2nd dose allowed[2]	Expected performance capability of unit	Field symptoms	Level III clinical remarks
1–70 cGy	1–14 days >14 days	149–80 cGy ?	Combat effective	Mild nausea and vomiting	Mildly decreased lymphocytes, platelets, and granulocytes— monitor lymphocyte count
Protracted[1, 3] 71–140 cGy	1–7 days >7 days	79–10 cGy ?	Partial/full decrement	Fatigability and weakness, greater nausea and vomiting	?

[1]All individuals are considered combat effective after the 1st dose, from Table 4-1, FM 8-10-7.
[2]Maximum total dose received should not exceed 1.5 Gy.
[3]This group should receive no further radiation exposure after 1st dose.

Summary of Session IV

Forward-Field Bioindicators for Dose Assessment: Possible Alternatives

William F. Blakely, Ph.D., and Thomas M. Seed, Ph.D.
Chairmen

Pataje G.S. Prasanna, Alasdair J. Carmichael, Narayani Ramakrishnan,
David A. Schauer[1], and Clive L. Greenstock[2]
Session Panelists

Armed Forces Radiobiology Research Institute, Bethesda, MD,
[1]Naval Dosimetry Center, Navy Environmental Health Center Detachment, Bethesda, MD,
and [2]Atomic Energy of Canada Limited (AECL), Chalk River, Ontario, Canada

- NATO Perspectives on Biological Indicators of Radiation Exposure
      Dr. Govert P. van der Schans
      TNO Prins Maurits Laboratory, Rijswijk, Netherlands

- Radiation-Induced Apoptosis in Human Lymphocytes
      Dr. Douglas R. Boreham
      AECL, Chalk River, Ontario, Canada

- Halo-Comet Assay
      Dr. Juong Gile Rhee, Jie Liu, Mohan Suntharalingam
      University of Maryland Medical School, Baltimore, MD

- Radiation Damage in the Hematopoietic System
      Dr. Lionel G. Filion
      University of Ottawa, Ottawa, Canada

- Automated Cytogenetic Assays in a Field Environment: Consideration of the Halo-Comet Assay
      Mr. Harry L. Loats
      Loats Associates, Westminster, MD

- Potential Use of *In Vivo* Electron Paramagnetic Resonance, Electron Spin Resonance (EPR, ESR) for *In Vivo* Dosimetry Under Field Conditions
      Dr. Harold M. Swartz
      Dartmouth Medical School, Hanover, NH

- EPR-Based Dosimetry and Its Present Suitability for Field Usage
      Dr. Arthur H. Heiss
      Bruker Instruments, Inc., Billerica, MA

Overview

The goals of Session IV were to evaluate the status of several alternatives to peripheral blood counts and prodromal symptoms for forward field radiation-dose assessment. The suitability of selected biochemical-based (DNA single strand breaks), cytogenetic-based (apoptosis, halo-comet), and biophysical-based (free radicals in solid matrix materials) bioindicators of radiation exposure was evaluated. The current status of fielding systems to automate the measurements was also evaluated. The session consensus was that no one assay is presently suitable for military use. It was also recommended that a research program be implemented to evaluate the development of a multiassay strategy characterized by *in vivo* evaluation studies, acute versus protracted radiation exposures, and critical comparisons between techniques with the greatest potential for both robust dosimetric capability and automation. Table 3 shows the classes of bioindicators, the type of assays, and the presenters.

Introduction

Suitable forward-field bioindicators should exhibit the following characteristics: (1) negligible post-sampling incubation, (2) rapid processing suitable for a high degree of automation and high throughput, (3) low-threshold, broad dose-rate, and variable radiation quality capability, (4) relatively noninvasive sample collection, and (5) equipment hardware for which components are or soon will be available.

Dr. Clive L. Greenstock's (AECL, Chalk River, Ontario, Canada) review of potential biomarkers of radiation exposure (presented in session I) provided excellent background. In addition, Dr. Govert P. van der Schans, chairman of the Bioindicator subgroup of NATO Research Study Group No. 23/Panel VIII (Assessment, Prophylaxis, and Treatment of Ionizing Radiation Injury in Nuclear Environments), provided a brief summary of related research efforts. He noted the limitations inherent to dose assessment methods involving lymphocyte counts and chromosome aberrations. Dr. van der Schans said the NATO group is studying the use of dextran sulfate to stimulate resting immune cells in peripheral blood as well as their dose-dependent cytogenetic-based response and the micronucleus assay. Further progress with cytological dosimetry is possible using premature chromosome condensation (PCC), chromosome painting using hybridization probes, and automated data collection and analysis using a metaphase finder. However, these techniques are inherently time consuming, subjective, and labor intensive. In addition, the analysis of more than five samples per day is problematical unless the assay is automated.

Presenters for Session IV were selected to permit an inspection and review of potential bioindicators from a broad spectrum or class of candidates (Table 4). All presenters were asked to address the status and practicality of fielding an automated dose-measurement system. The seven presentations in Session IV were each limited to 20 minutes and reflected an intent to bridge the gap between science and industry. Representatives from two science-application companies, Mr. Harry L. Loats (Loats Associates, Westminster, MD) and Dr. Authur H. Heiss (Bruker Instruments, Inc., Billerica, MA), gave presentations on automated cytogenetic assays and EPR-based dosimetry, respectively.

An open discussion evaluating and comparing the various biomarkers for radiation exposure followed the presentations.

Table 3. Class of bioindicator, type of assay, and presenter.

Class	Assays	Presenter(s)
Biochemical	DNA single-strand breaks	van der Schans
Cytogenetic	Apoptosis	Boreham; Filion
	Halo-comet	Rhee; Loats
Biophysical	Free radicals in solid matrix (EPR)	Swartz; Heiss

Possible Alternative Bioindicators for Dose Assessment

Biochemical

The use of an immunochemical-based method to detect radiation damage to DNA appears to offer the possibility of providing the necessary requirements of a fast, simple, direct biological indicator using a blood sample. The assay involves using specific monoclonal antibodies against DNA single-strand breaks attached to the surface of multiwell plates. Small volume blood samples ($3\mu l$) are diluted in alkali to unwind the DNA at single-strand breakage sites. The blood samples are neutralized and sonicated to release single-strand fragments. The fragments as antigens are allowed to form complexes with the coated monoclonal antibodies; and the complexes are conjugated with alkaline phosphatase and incubated with an FITC-labeled substrate—the fluorescent intensity of which is directly proportional to the amount of single-strand fragments which in turn is proportional to the radiation exposure. The assay requires only small samples, takes 1–2 hours, does not require cell culture, and has a lower limit of detection of ~0.2 Gy.

Because of rapid DNA repair, *in vivo* samples must be collected immediately after exposure (<1 hour) or a much lower sensitivity is achieved—since DNA strand-break damage is rapidly repaired. In a practical situation (the far-forward field), the reliable lower limit of detection is probably 1–2 Gy—taking into account the wide variation in individual radiosensitivities and the controls' baseline level DNA breaks. More *in vivo* tests are required to validate this promising potential biodosimeter and to compare acute versus chronic or protracted exposures as well as the varying effects that result from differences in radiation quality.

Cytogenetic

Apoptosis Assay. Dr. D. R. Boreham addressed the possibility of using apoptotic death in human peripheral-blood lymphocytes as a biological dosimeter and reviewed the steps involved in human lymphocyte apoptosis—a rapid, sensitive, reproducible biological response to low-dose radiation exposure. Although most research involves assays requiring cell culture, the kinetic changes involved in the process of apoptosis offer the possibility of using apoptosis as a potential biological dosimeter, provided that a blood sample is obtained within hours of human exposure. The characteristic DNA fragmentation is detected using either the comet technique, by *in situ* terminal deoxynucleotidyl transferase (TdT) assay, or the fluorescence analysis of DNA unwinding (FADU) assay. For *in vitro* exposures the induction of apoptosis is proportional to dose; the lower limit of detection is ~ 0.05 Gy. Overall radiation-induced DNA damage is repairable with a half-life of ~1 hour. After about 4 hours postexposure, the ordered DNA fragmentation

Table 4. Selected list of alternative bioindicators.

Clinical chemistry kits for low molecular weight products in body fluids	• Electrolytes, creatine, taurine
	• Prostaglandins, enzymes, metabolites, nucleotides
	• Molecular/chemical biosensors
	• Immunochemicals, antibody tests
Immunochemical tests	• Cytokines, lymphocytes, hormones
	• Membrane markers
	• Cyclins, integrins, etc.
Genetic engineering	• Differential display (multiple gene expression)
	• DNA probes (FISH, PCR, RFLP, mutation spectral analysis)

characteristic of active apoptosis becomes apparent and increases over 24 to 48 hours.

In-vivo exposures require the removal of lymphocytes from the body as soon after exposure as possible (within hours), otherwise apoptotic cells will disappear as a result of phagocytosis. One technique that can potentially measure apoptosis is flow cytometric-based analysis of lymphocyte plasma membrane changes—the early and critical events in apoptosis. Dr. Lionel G. Filion emphasized (see session II manuscript) the potential benefits from the use of flow cytometry-based methodology. Clearly the use of multiple parametric endpoints derived from several distinct cell populations should provide a significant improvement in clinical dose assessment.

Because individual radiation-induced apoptotic responses vary greatly, this assay measures biological radiosensitivity rather than physical absorbed dose. Apoptosis has considerable potential as a future biodosimeter and is amenable to the development of a fast, automated, clinical kit to test for radiation exposure using a small (0.1 ml) blood sample; however, *in vivo* (animal or radiotherapy patient) testing is urgently required.

Halo-Comet Assay. Dr. J. G. Rhee presented results from a modified version of the "halo assay," designated the halo-comet assay. The halo-comet assay appears to quantify alterations of an individual cell's DNA organization. Dr. Rhee used two *in vitro* cultured mammalian cell lines for these studies. Agarose gels containing single cells, which had been exposed to different doses of x-rays and various repair intervals, were prepared on glass slides. Following electrophoresis (pH-7 conditions), the cell preparations were stained with propidium iodide. The dye was excited, and image data acquisition detected with fluorescent light (excitation: 545 nm, emission at 580 nm). Data were analyzed by image analysis. A linear increase of up to 6 Gy in the "amount of DNA pulled from the nucleoids" was suggested by the results. Significant differences between 0 and 0.5 Gy induced damage, with no repair, suggests that this assay is sensitive enough for triage purposes. The analysis of residual damage after 30 min of repair following exposure to 2 and 4 Gy of

x-rays indicated a significant difference between irradiated and control samples. This was evident even after 1 or 6 days of repair, indicating that some damage could still be measured after a time lapse. Dose-dependent alterations following fractionated doses (2 Gy x 3 days) were also observed after 5 days of repair following the last fraction. These results should also be confirmed by *in vivo* studies.

The assay is rapid and can be automated by image analysis, which was the subject of Harry L. Loats' presentation. Loats presented a system based on the halo-comet assay designed to automate cytogenetic assays in a field environment. The system components are based on the existing automated systems produced by Loats Associates, Inc., for clinical and research laboratory applications. To accommodate the throughput requirements inherent in the military-field scenario, a parallel processor and multichannel design was presented with characteristics including (1) robotic slide selection and delivery system, (2) automated cell finder using non-fluorescent lighting, (3) a new technique for image-based low-light level range extension to provide dynamic range extension for capturing the head and tail of the halo comet in the same image, and (4) a digital method to produce composite images from a series of closely spaced images neighboring the plane of best focus.

The system uses off-the-shelf and proven hardware and software, can be operated by non-specialist personnel, and would be applicable to both forward-field and rear-support echelon facilities.

The detection system for dose assessment using the halo-comet assay has distinct possibilities. It likely will exhibit a broad dose range and can potentially be performed with blood, making the sample collection relatively noninvasive. A halo-comet assay system has the potential to handle a large number of samples using automated image analysis; its relatively short assay time is a major benefit over alternative biomarkers. The possible applicability of this assay for use in human dose-assessment applications needs to be explored with animal models and radiotherapy patients. However, studies addressing the effect of individual variations in radiosensitivity

and effects from confounding factors (e.g., prior exposure to genotoxins, dose protractions, and different exposure scenarios) need to be performed.

Biophysical

Free Radicals in Solid Matrix Material. Free radicals in solid matrix can be measured by electron paramagnetic resonance (EPR) spectroscopy, a well established and accepted technique for determining absorbed radiation in cases of accidental radiation exposure. Dr. Harold M. Swartz and Dr. Arthur H. Heiss presented aspects of this subject. Dr. Swartz provided results from EPR spectroscopy using tooth enamel and bone dosimetry, although several alternatives (clothing buttons, nail clippings) are presented in his manuscript. Swartz also emphasized the need to investigate other biological sample systems for EPR dosimetry measurements. The workings of the EMS 104 portable EPR analyzer, designed specifically for dosimetry purposes and now in use in many clinical settings, was presented by Dr. Heiss. In addition to using it for alanine dosimetry, Heiss presented additional potential uses for this EPR instrument, including the red blood cell receptor assay being developed at AFRRI by Dr. Alasdair Carmichael using spin-labeled insulin. Application of the Bruker EMS 104 for use in military forward-field applications was also discussed.

While EPR-based dosimetry has several desirable features, there are also significant limitations. The requirement to either assess in advance each individual's radiosensitivity in relation to their solid matrix material (teeth enamel, bone, etc.), or to establish a dose response calibration curve from a test sample, restricts practical use of this approach for military applications. This second approach requires a radiation source in the far-forward field laboratory; however, use of an isotope-based gamma-ray source for this purpose is not advisable in battlefield applications.

Other Bioindicators to Consider

Several other potential bioindicators were identified that may have merit as alternative biomarkers for military forward-fielding as dose assessment assays

(Table 4). Unfortunately due to time constraints, discussion of these assays was limited.

Discussion

Accomplishments

Advances in biotechnology, digital imaging, and data handling and analysis can effectively eliminate manual analysis procedures for biological dose assessment—which are subjective, time-consuming, and labor-intensive. Parallel development of equipment with improved fieldability characteristics (compact, rugged, portable multiplexing technology to dramatically increase throughput with fewer moving parts) coupled with these radiation bioindicator assays is feasible. For example, the automation of cytogenetic assays (metaphase finder, image processing, chromosome aberration/micronucleus/fluorescence *in situ* hybridization (FISH)/premature chromosome condensation (PCC scoring)) were demonstrated. There is a need to develop alternative *persistent* damage endpoints (halo-comet, membrane markers, *in vivo* ESR biological dosimetry) that do not require cell culturing. These assays require tests of individual radiosensitivity with appropriate *in vivo* validation.

Remaining Research Questions

These findings, along with contributions from workshop participants, permitted a delineation of relevant remaining research questions. Several significant questions that were identified include the need to (1) improve existing assays or develop new assays to make the process faster, simpler, more direct, and definitive, (2) look for mechanisms underlying intraindividual and interindividual variability, (3) search for a radiation-specific response or signature, (4) carry out interlaboratory comparison, standardization, and validation, (5) overcome the problem of needing prior knowledge of an individual's radiation history (dose-rate, LET, etc.) and baseline responses, and (6) establish necessary criteria (and weigh the advantages and disadvantages) of a biological *dose* indicator versus a biological *effect* indicator.

Recommendations

The consensus of the session was that none of the dose assessment biomarkers has all of the features representing the "ideal" assay; alternatively, a multi-assay approach represents the best design to meet mission requirements. To accomplish the goal of fielding a practical dose-assessment assay, a research program should (1) concentrate future experiments on *in vivo* testing (animals or radiotherapy patients), (2) compare results from acute *versus* protracted exposures, (3) move toward standardized protocols using the same *in vitro* cell lines (preferably human), (4) carry out critical comparisons between the techniques with the greatest potential, and (5) develop combinations of assays performed sequentially or in parallel (preferable).

Executive Summary

Operational Effectiveness of Exposed Personnel

The consensus was that personnel receiving radiation of 1.50 Gy or more should be evacuated to the rear, while most personnel can be returned to duty even after exposure to a "significant" amount of radiation, up to 1.50 Gy (free-in-air). All irradiated individuals will be at increased levels of susceptibility to morbidity and mortality from other illness and injury (e.g., communicable and infectious disease, mechanical and/or thermal trauma, etc.). Up to 30% of personnel receiving exposures approaching 1.50 Gy will be too clinically ill to return to duty, and will require evacuation to an echelon III or higher-level facility. After recovery from the acute effects, many will experience fatigue and weakness of varying degrees for up to several weeks, although they otherwise will be able to continue their duties. The decision of whether to leave in place either individuals or units that have been exposed to radiation is ultimately an operational—not a medical—decision. (But see below, final paragraph of this section.)

An important point: The general consensus was that clinical symptoms and hematological indicators are inadequate for initial triage in a forward-field setting, given the rapid time constraints, limited personnel and equipment, and potential use of prophylactic emetics in this situation. First-line triage and dose assessment would be better served by the application of physical dosimetry. Clinical, serial hematological, and other biological parameters will be more useful in secondary triage processes and in rearward medical facilities.

During the discussion Dr. Vic Bond, who was one of the physicians involved in caring for the Marshallese Islanders exposed to fallout in 1954, made an important point regarding medical evaluation of radiation-injured patients. The clinical presentation and course, *not* the physical dosimeter's reading or other laboratory parameters, will determine the priority and nature of treatment.

In interpreting Dr. Bond's comments and his single view graph (Fig. 2), the problem of placing reliance on physical dosimetry for medical assessment purposes stems from inherent differences in slopes of the dose-response functions for the primary biological endpoints of interest, namely fatality and injury severity (i.e., injury to organ systems, tissues, and selected target cells). Physical dosimetry best serves the narrow, steeply-sloped "probability of fatality" dose function (Fig. 2.a), while serving poorly the broader, initially shallow-sloped "severity of injury" function (Fig. 2.b.) The integration of the two functions (fatality vs. injury severity) tends to an extremely narrow window of expression of fatal-type responses, relative to the overall extent of injury severity (Fig. 2.c).
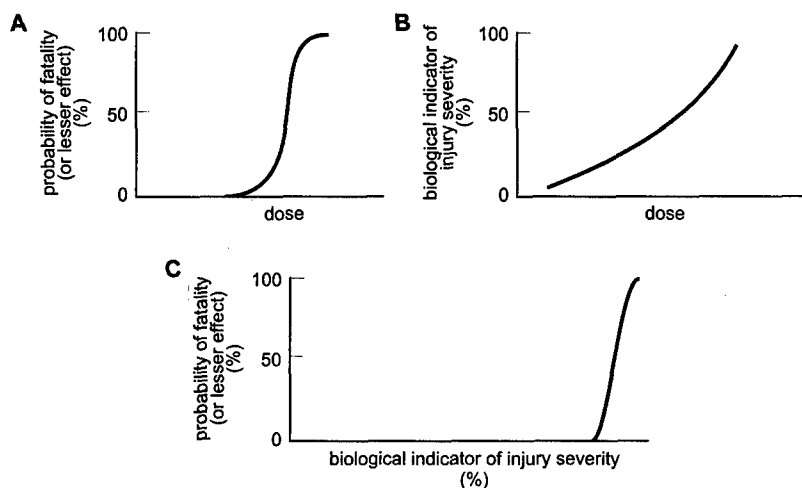

Fig. 2. Differences in slopes of the dose-response functions for the primary biological endpoints of interest.

As a result, the major fraction (and degree) of the injury-severity response appears to be poorly represented at the sublethal response levels. Accordingly, the use of physical dosimetry for medical triage would be based on a fatality probability function, rather than on an injury severity function, and thus would tend to ineffectively represent the degree of treatable injury, measured more appropriately by biomedical methods.

To summarize: physical dosimetry is required in triage at forward medical facilities, given the rapid (24 hr.) time constraints and personnel and resource limitations, to determine if exposure was high enough (1.5 Gy or more) to require evacuation, and to determine the probability of lethality. Biomedical response indicators become necessary, primarily at rearward facilities, to determine the severity of (survivable) injury and the optimal clinical management of the patient.

Operational Effectiveness of Personnel with Multiple Exposures

There were three major consensus conclusions regarding this topic: (1) No animal model completely predicts the effects of either acute, protracted, or multiple radiation exposures in humans; (2) fatigue and weakness are cumulative; and (3) physical dosimetry is required for personnel at risk of a second exposure.

The models used to address this question need further experimental validation in terms of dose-rate and fractionation intervals. Large animal data have been used to develop human LD_{50} models, but the correlation of survival curves between species let alone between large animals and man, is not always constant. Marrow kinetics are certainly related to mortality, and the pathologic sequelae that contribute to a fatal response; though there are probably other factors besides marrow damage, even at this dose range, that influence lethality.

Fatigue and weakness start at only 1 Gy, and there is no apparent repair coefficient or fractionation effect; i.e., the effects from multiple doses are cumulative. This also appears to be independent

of dose-rate effect, as well as for the interval between discrete prompt doses. Emesis, however, is affected by factors of dose rate and fractionation. In terms of marrow kinetics, repair does take place; the degree to which it occurs is dependent on radiation dose, dose rate, radiation quality, the volume of marrow irradiated, and the species being irradiated.

Estimating Exposure in Personnel Who Received Antiemetics Before or Shortly After Exposure

The three symptoms currently used to clinically assess the degree of radiation exposure are nausea, vomiting, and diarrhea. Use of antiemetics before or shortly after known exposure clearly masks the response of the exposed person to radiation and could actually improve operational effectiveness. Even so, there is individual variability in the effectiveness of antiemetics. Although multiparameter hematological indices are probably not affected by the use of antiemetics, there is no reliable clinical or laboratory bioassay at echelon I and II facilities capable of exploiting this fact. Fatigue and weakness, even if unaffected by antiemetics, are too highly subjective to provide a reliable, reproducible, quantifiable, and accurate measurement of exposure. As mentioned earlier, physical dosimetry remains at present the only reliable tool for exposure estimates, regardless of whether personnel have received antiemetics. Accordingly we recommend the following procedures:

1. Obtain a base level complete blood count and differential if exposure is anticipated.

2. Provide physical dosimetry, readable at this level, and make available for 100% of the troops.

3. Perform serial blood counts as soon as possible postexposure.

4. Consider the above procedures carefully before ordering administration of prophylactic antiemetics. The decision to administer these drugs must be made by the commander;

drugs should not be issued until exposure is likely and should not be taken until directed.

There are three areas of development for biological dose indicators that may become available at echelon I and II facilities in the near future: (1) develop a hand-held, durable electronic blood-cell counting device capable of performing either single or multiple blood cell (lymphocyte) depletion assays; (2) find, if possible, more reliable, precise, and quantifiable means of defining fatigability and weakness (which would also serve to better support radiation associated performance decrement evaluation models); or (3) use hardened, automated, and sophisticated cytogenetic (or other) assays (see final paragraph).

The application of multiparameter hematologic assays as well as other assays in echelon III facilities and beyond is critically important. Assays at this level serve to confirm initial exposure estimates made by physical dosimetry, assess the extent of injury to the lymphohematopoietic system, and provide the basis for therapeutic management.

One encouraging note is that there are near-term technologies that will provide additional bio-assays (besides serial lymphocyte and multi-parameter blood counts) at echelon III facilities. These include biochemical assays (radiation-induced single strand DNA breaks), biophysical assays (electron paramagnetic resonance (EPR) analysis of free radicals in solid matrix materials), and cytogenetic assays (apoptosis, halo-comet assays.) At present, the chief drawbacks for most of these techniques are the high level of skill required to run these generally time-consuming and labor-intensive procedures (with resultant low throughput) and their current unsuitability for field conditions (harsh environment, mobility, ruggedness). Also, further research into individual radiosensitivity, *in vivo* validation, results of acute vs. protracted exposures, etc., is required. With the near-term development of automation and field hardening, some of these procedures may become useful options— and perhaps can be used as far forward as echelon II facilities.

Appendices

Appendix A

Session I

Operational Capabilities of Army Forward Deployed Medical Units and Supporting Laboratory Facilities

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This lecture will cover the operational capabilities of Army forward deployed field medical units and their organic or supporting laboratory facilities. A basic description of the Army's current combat health support doctrine will be described and will include an overview of the operational medical and laboratory capabilities of current echelon I and echelon II medical units (e.g., the forward surgical team, the forward support medical company, and the battalion aid station). We will also outline the operational capabilities of future laboratory units/facilities that will be modernized under the Army Medical Department's Medical Reengineering Initiative (MRI) and programed incremental change programs. This overview will conclude with an in-depth analysis of the operational capabilities of the current Theater Army Medical Laboratory (TAML) and the MRI redesigned Area Medical Laboratory, which will replace the TAML. The focus of this discussion will be on the unique nature of the Area Medical Laboratory which is designed to deploy in a split based mode and will have the capability to send small tailored teams forward on the battlefield to interface with units detecting and/or collecting biomedical, environmental, and suspected nuclear, biological, and chemical (NBC) threat samples/specimens. Of particular interest will be the Area Medical Laboratory's mission of providing in-theater, field-laboratory confirmation for endemic diseases and NBC threat agents.

Overview of Current Organization and Operational Policy for Combat Health Support

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"The battlefield of the future will be characterized by increased lethality, speed and depth. Resources have diminished resulting in a smaller Army. Moreover, the criterion for success is to win swiftly with minimum casualties.

"The soldier is still the key to American victory in war. Quick decisive victory requires land force dominance. To achieve land force dominance, the Army must continually field high payoff technologies that support the five objectives of our modernization vision: quickly project and sustain forces; protect those forces; win the battlefield information war; conduct precision strikes; and dominate the maneuver battlefield."

Former Chief of Staff of the Army General Sullivan has precisely outlined the U.S. Army's concept for entering the 21st century with smaller, yet through the use of technology, more agile, more lethal, and more dominant forces on the battlefield.

Through the use of technology, especially communication technology, Army medical personnel will locate casualties faster and more precisely, and will

start resuscitative treatment earlier. First aid will remain self-aid and buddy aid. With the introduction of the combat lifesaver almost 10 years ago, first aid has advanced to include the simple management of airway blockage, the prompt control of hemorrhage, splinting of fractures, and protection of wounds. The combat lifesaver is a fighting soldier whose first responsibility is to engage in combat. If his commander so elects, he will also provide enhanced first aid prior to medical treatment by the combat medic for comrades-in-arms who have become casualties.

The combat medic is the first of the medical treatment personnel to manage casualties. There is some overlap between the capabilities of the combat lifesaver and the combat medic. The combat lifesaver is a combat soldier; the combat medic is the first medical treatment person in the echelons of medical care. The combat medic initiates true resuscitation of the more severely wounded casualties, and after first practicing the ABCs of initial trauma care, prepares the casualty for evacuation to the ambulance collecting point.

Transportation from the ambulance collecting point to the Battalion Aid Station (BAS) occurs as quickly as possible. A physician is assigned to the BAS. The practice of medicine at this level is characterized as "tailgate medicine," with medical personnel working out of a chest from the back of a vehicle, frequently without establishing a shelter, and without the benefits of x-ray or laboratory support. There is no capability for holding patients; the patients are either returned to duty if they can continue the battle; or they are evacuated to the next echelon of medical care for further treatment.

The first echelon consists of the combat medic in the combat company (usually one combat medic per platoon of about 40 soldiers), the casualty collecting point (or the ambulance exchange point), and the BAS. Echelon I includes a physician, a physician's assistant and six enlisted personnel at the BAS and three or four enlisted medical personnel in the maneuver (combat) companies of the combat battalion. Echelon I has the responsibility for the initial medical treatment of casualties.

Echelon II consists of the forward medical company located in the vicinity of the trains area of the combat brigade. Patients being evacuated from echelon I are either transferred to echelon II (usually by ground) or by helicopter to a forward hospital. There is very limited laboratory and x-ray capability at the medical company, nor is there much holding capacity. The beds consist of typical cots, and are meant only for patients who will be ready to return to duty and fight after 3 to 4 days of rest that include medications, antibiotics, and intravenous fluids. The principal purpose of the medical company is to provide outpatient services, to be a stopping place for patients being evacuated to a hospital by ground ambulance, and to provide assistance to the collocated forward surgical team. The laboratory consists of one person and a few simple items of equipment that permit routine blood counts, routine urinanalysis, gram stains and dipstick technology. The x-ray facility consists of one small field machine and one technician; the primary use being extremity and chest x-rays. If a patient is not expected to return to duty within the 3- to 4-day limit, then the patient should be evacuated promptly to echelon III. Both echelon I and II facilities must remain capable of frequent moves; it is difficult, if not impossible, to move with patients. Echelon I may move more than once during the day, depending on the advance of the combat forces. The medical services must keep up with the line if they expect to adequately and promptly care for casualties. In Operation Desert Shield/Storm (ODS/S) the division of medical forces were constantly moving to keep up with the combat forces.

Brief review of two terms: Killed in Action (KIA) and Died of Wounds (DOW). KIA is used to describe those casualties who die from their wounds prior to arriving at a field medical facility. The first (most far forward) medical facility is the battalion aid station at echelon I. That facility is where the first physician is located. Through WWI, WWII, Korea, Vietnam, and ODS/S, the KIA rate has been consistent at about 20% of battle casualties. In contrast, the DOW rate has decreased from 9% in WWI to less that 1% in ODS/S. DOW denotes those casualties who arrive at the battalion aid station alive, but who subsequently die in any medical facility. Hospital care of casualties has improved to the

point of essentially total survival in the medical system; while the far forward death rate has remained unchanged. Good studies reveal about one-third of those listed as KIA had wounds which should not have been lethal if the response of the combat medic had been faster and his armamentarium improved.

Triage occurs at every level and is a continuing process. Triage broadly defined is the sorting of patients into several categories for treatment, evacuation, and management purposes. As a patient's condition changes he may need to be retriaged into another category. Triage categories consist of those patients who urgently need immediate treatment, such as maintaining an airway or controlling hemorrhage; those whose treatment can be delayed without significant deterioration of their condition; and those whose injuries give them a poor chance of surviving. Proper triage assures the utilization of scarce resources for those who have the greatest opportunity for survival. In the nuclear environment, initial triage will have to be based on the casualties' wounds; since the lack of an accurate radiation dosimeter prevents triage based only on radiation dose. With the radiation-only injury, the medical triage decision will have to be based on the ability of the soldier to return to effective combat duty. Initial symptoms will be the guide for this medical decision.

It is important to recognize that making fit-for-duty decisions based only on symptoms, without the benefit of an accurate radiation dose, may return personnel to duty whose health could be jeopardized by further duty. Likewise, others may be evacuated who could have continued the mission. The decisiveness of the combat may dictate that anyone capable must remain as the line commander directs. In general, vomiting soldiers make less than desirable combat soldiers.

Triage is also extremely important prior to making medical-care decisions. Only those with a reasonable hope of survival should be subjected to extensive surgery. Surgery must be performed promptly before the effects of radiation prevent healing. Radiation combined with other injuries quickly changes a casualty from delayed treatment to expectant. As noted previously, an accurate

dosimeter will be invaluable for appropriate triage at both echelon I and echelon II.

Many years and multiple meetings were required for the development of NATO Standardization Agreement (STANAG) 2866, "Radiation Injuries and Effects of Radiation on Operational Effectiveness of Personnel." Although this is no longer a STANAG, it is useful guidance for medical personnel who must make triage decisions on the nuclear battlefield. With a known dose of radiation, the medical officer can accurately advise the commander on the medical consequences of the commander's decisions.

Bio-triage of radiation injury is not possible at echelon I since there is no laboratory capability. Physical dosimetry is the only possibility, unless the physician at the battalion aid station is satisfied with using clinical symptomatology. Lymphocyte counts are available at the echelon II medical company; however, holding patients for 48 hours to observe changes in lymphocyte counts is not practicable. The medical companies have limited cots (usually not more than 40). The need for cots for other patients precludes retaining patients for observation. Thus, triage for radiation-injury-only casualties is difficult without the prompt knowledge of the radiation dose. The wrist-watch dosimeter is not adequate as a basis for life or death decisions. For combined-injury patients, initial triage decisions will have to be made on the basis of the other injuries, with the radiation injury ignored unless the casualty sustained a non-survivable radiation dose as evidenced by prompt or early clinical symptoms.

Combat Developments at the Army Medical Department (AMEDD) Center and School maintains a large patient-condition model for determining workloads, logistical support, and personnel-resource requirements for tactical scenarios. Included in this model are five radiation-casualty conditions, ranging from casualties with radiation exposure and minimal symptoms who are evacuated to a hospital for evaluation with return to duty in 2 days, to the other extreme, casualties who die from radiation injuries in approximately 1 week. Current combat scenarios suggest that in the event

of nuclear device deployment, radiation casualties will be evacuated to hospitals, at least for the initial evaluation, since divisional medical units are poorly equipped to evaluate patients with only radiation injuries.

The use of oral antiemetics is currently being studied. To be most effective, oral antiemetics should be ingested prior to a nuclear episode. Like pyridostigmine for pretreatment for nerve agents, oral antiemetics need to have FDA approval before they are issued to soldiers. In addition, specific doctrine must be written to guide field commanders on when to issue the tablets and when to give the order to start taking the medication. Current stockage of injectable antiemetics in the medical equipment sets is insufficient to manage radiation casualties.

Each unit in the nuclear/biological/chemical (NBC) environment should be well acquainted with decontamination procedures for chemical warfare contamination; and all medical units have medical equipment sets available for decontamination of casualties. Fortunately, radiation decontamination is not as involved as chemical-warfare decontamination. Removal of clothing, brushing or washing of hair, and washing of exposed skin is usually adequate. Iodine and chelating agents are not stocked in the field sets; until now they have not been considered essential. In addition, the physicians in the field are not usually trained in the use of these agents. No policy exists for stocking or issuing chelators or blockers.

Combat stress may be as great a casualty producer as radiation injury. Seventy-five to 90% of combat stress casualties can be held at a separate facility within the division and returned to duty within 3 to 4 days. The U.S. Army has become astute in the management of combat stress casualties having learned its lesson in Korea on how devastating these losses can be.

In a well trained, well equipped U.S. Army, a 10 to 20% casualty rate for radiation injuries should be a viable estimate. Personnel with exposure below 150 cGy should not be significantly degraded and in most cases should be able to continue the mission with minimal medical support. The problem of

accurate dosage, as previously mentioned, hinders accurate triage and casualty management.

The management of acute surgical trauma in the combat environment is well documented. Likewise, thermal-injury management has been well documented. Major armored combat produces an incident rate of 10% for burn casualties. Inhalation injuries and burns of the head and neck are the most acute problems associated with thermal injury. In combined injuries, the prognosis is worse than for either injury alone. Subcasualty producing exposures of radiation, combined with a minor injury, neither of which alone would require hospitalization, could then require hospitalization.

United States Air Force Field Laboratories Capabilities

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The United States Air Force (USAF) deployable medical field laboratory capabilities are comprised within the air transportable hospital (ATH) system. This system consists of personnel and equipment capable of providing limited laboratory support in the areas of hematology, chemistry, urinalysis, and microbiology. The field medical laboratory is staffed according to bed capacity and specialty services availability (e.g., ICU, Gyn, Surgery). Laboratory officers are typically deployed to the larger ATHs (10 beds). Senior noncommissioned officers manage the smaller ATHs. In the context of forward field medical triage for radiation exposure, the USAF deployable medical field laboratory can provide a number of services to assist in diagnostic biodosimetry and clinical support for medical management of radiation casualties. Hematological assays include platelets, red and white cell counts (manual and automated), and white-cell differentials (manual). Chemical analyses include standard serum chemistries such as glucose, BUN, and electrolytes. Microbiological review includes gram stains, very limited bacteriologic culturing of various body fluids and wounds, and direct "wet"

preparation reviews for parasites and fungi. No field capability currently exists for the recovery and identification of viruses, micro-organisms commonly associated with biological warfare scenarios, or tuberculosis. Equipment to support these tests include refrigerators, centrifuges, incubators, hand refractometers and microscopes. Packed red blood cells (type O) are expected to be available for the emergent situation. Serological testing, if available, is very basic. A review of current and proposed capabilities and equipment on the table of allowances for Air Force deployable laboratories will be discussed.

Status and Limitations of Physical Dosimetry in the Field Environment

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Introduction

Military personnel are exposed to ionizing radiation during routine peacetime operations. External exposures are monitored using National Voluntary Laboratory Accreditation Program (NVLAP) accredited thermoluminescent (TL) dosimetry systems. These systems are capable of determining a wide range of doses (up to 5 Gy) from a variety of radiation types and energies. However, readers for most of these systems are centrally located and are not appropriate for use in field environments. Therefore, tactical dosimetry systems for use in wartime or contingency operations are maintained by the U.S. Army (AN/PDR-75) and the U.S. Navy (DT-60). This paper will describe the NVLAP accredited and tactical dosimetry systems in the Department of Defense. Strengths and weaknesses

of these systems and possible future systems will also be presented.

External Dosimetry Systems in the Department of Defense

Occupational Dosimetry. Processors of personnel dosimeters used to monitor radiation exposures received by workers under Nuclear Regulatory Commission (NRC) licenses are required to be tested and accredited through the NVLAP (10CFR20) [1]. Administered by the National Institute of Standards and Technology (NIST), the NVLAP accredits public and private testing/calibration laboratories. Accreditation is a formal recognition of a laboratory's competence to carry out specific tests or calibrations. Accredited laboratories have demonstrated their capabilities through proficiency testing and an on-site assessment.

Each service maintains NVLAP accredited dosimetry systems. The U.S. Army Ionizing Radiation Dosimetry Center at Redstone Arsenal and the U.S. Air Force Dosimetry Center at Brooks Air Force Base use Panasonic TLD systems. These systems consist of the UD-802 dosimeter (225,000), the UD-710 (9), and the UD-716 (5) readers. The U.S. Navy maintains two dosimetry systems: Naval Reactors Dosimetry System administered from Puget Sound Navy Shipyard uses calcium fluoride bulb dosimeters (115,000) and CP-1112 readers (405); and the Naval Dosimetry Center at the National Naval Medical Center uses Harshaw lithium fluoride dosimeters (95,000) and 8800 (4) readers.

Tactical Dosimetry. The U.S. Army has fielded the AN/PDR-75 high-range personnel dosimetry system. It monitors neutron- and gamma-ray doses in the range 1–999 cGy with overrange indication and is intended to assist commanders in making operational and medical decisions by providing timely dosimetry data. The system utilizes radiophotoluminescent (RPL) glass and silicon diodes as the radiation sensors in a wrist band style dosimeter (DT-236). These are issued to each individual in a combat role. Currently, there are approximately 280,000 ready for issue. The

dosimeter reader (CP-696) contains a flashtube and photocell for RPL glass evaluation. A separate circuit is used to measure the forward bias of the silicon neutron sensor. Readers are issued to company-size units for use in the field. Dimensions are 14 x 8 x 8 inches; weight is 24 lbs. They operate on 21–30 V dc and 1.5 A.

Extensive testing of this system in a simulated fallout environment was conducted at the Armed Forces Radiobiology Research Institute (AFRRI). Experimental design consisted of irradiating 10 dosimeters in a ^{60}Co irradiator at 2 cGy/min to a daily dose of 50 cGy and a total dose of 500 cGy. Figure 1 shows the results for dosimeters read at 15 min, 1, 2, and 24 hrs postirradiation. Results included a strong growth of reading in the first hour (15 minute reading was only 50% of delivered dose); the 1-hour reading was within 15% of the final reading. Growth was essentially complete at 24 hours. The final reading was approximately 5–8% low and the standard deviation was about 2–3% [2].

The DT-60 system consists of over 500,000 RPL glass dosimeters to monitor high-energy photon dose in the range 0–600 cGy and over 2,000 compact readers (CP-95). The RPL signal from the DT-60 also has a time dependence like the DT-236.
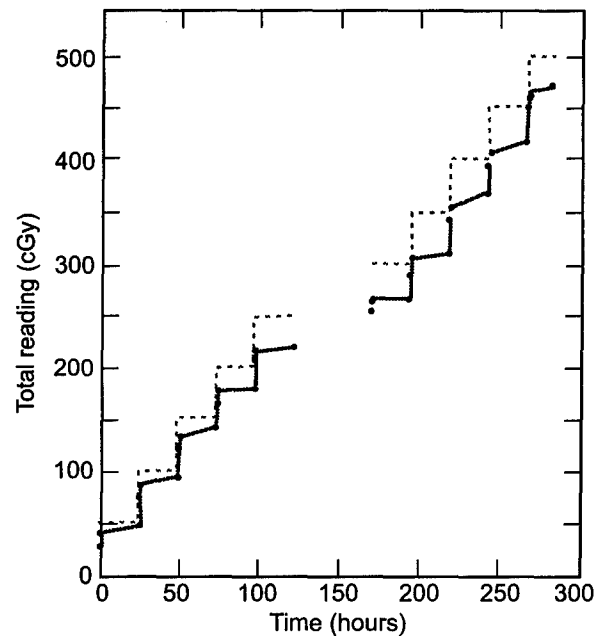

Fig. 1. Response of DT-236 dosimeters exposed to 50 cGy per day at 2 cGy/min for 2 weeks. Squares/step-ramp: delivered dose. Circles: average of 10 dosimeters read at 15 min, 1, 2, and 24 hr after irradiation. The gap at the center of the graph represents a 2-day break when no irradiations were done.

Strengths and Weaknesses of Dosimetry Systems

The strengths and weaknesses of both systems are summarized in table 1.

Table 1. Strengths (+) and weaknesses (-) of occupational and tactical dosimetry systems.

	Occupational	Tactical
Number of dosimeters	+ (>400,000)	+ (>800,000)
Number of readers	+ (>400)	+ (>5,000)
Processing	- (Centrally located and not ruggedized)	+ (Field deployable and ruggedized)
Operational range	+ (10 mrem to 5 Gy and higher)	+ (0 to 20 Gy)
Response characteristics	+ (Calibrated using high-energy photons)	+ (Accurate to within 5–8% at 24 hrs, however, 50% lower 15 minutes PI)
Read mechanism	- (Thermoluminescence -destructive)	+ (Photoluminescence -nondestructive; provides a permanent record)

Future Dosimetry Systems

AN/UDR-13. The AN/UDR-13 is intended to replace the IM-93 high dose-range pocket ionization chamber. It is a direct reading dosimeter that measures dose rates from 0.1 to 999 cGy/h ($\pm 20\%$) and total gamma plus neutron dose from 1 to 1999 cGy, with audible and visual alarms. Dimensions are 4 x 2.5 x 1 inches, weight is 8.5 ozs. It is powered by 4 AAA alkaline batteries. Current policy is to issue 1 AN/UDR-13 to each platoon.

Teledosimetry. Teledosimetry provides a two-way communication link that networks radio transmission of dosimetry data and provides remote display of dose status. It uses spread-spectrum transmissions for high transmission reliability at low signal levels. This technology has been used as a dose control and mitigation tool in high radiation fields encountered during steam generator inspections in civilian and naval reactors.

Conclusions

Large numbers of dosimeters can be provided by both occupational and tactical dosimetry systems. Accurate and precise measurements over a wide range of doses can also be provided by both systems. However, occupational systems are centrally located and are not ruggedized; therefore, they are not appropriate for field use. Tactical dosimetry systems are ruggedized and can be used in the field environment. Future systems should incorporate telemetry capability to provide real-time dosimetry results that can be remotely monitored. Dose mitigation can also be accomplished with this technology.

Acknowledgments

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Selection of a Drug for Prevention of Radiation-Induced Nausea and Vomiting

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Abstract

Project Group 29 (PG-29) was authorized in June 1989 by the NATO Army Armaments Group (NAAG) to evaluate Serotonin-Type 3 (5-HT$_3$) receptor antagonist antiemetic drugs and to make a recommendation concerning their use as a self-administered means to prevent nausea and vomiting—the primary performance-degrading effects likely to result from operational exposure to ionizing radiation.

Both candidate drugs were found by their manufacturers to significantly reduce the proportion of individuals who experience nausea and vomiting after therapeutic exposure to radiation, and to delay the onset of these effects in those individuals in whom complete protection cannot be achieved. Both drugs have remarkably few side effects, all of which are infrequent and minor in nature. Work sponsored by PG-29 confirmed that the drugs do not impair the performance of physiologically and

psychologically demanding tasks, including high-fidelity flight simulation. Both drugs have an excellent margin of safety, produce no acute or delayed toxicity, and have no potential for abuse. Both drugs are compatible with nerve agent pretreatment and therapy drugs and have been assessed as compatible with the other medications used in service personnel, including the drugs and anesthetics used in emergencies. Kytril® and Zofran® have been approved by civilian regulatory and licensing authorities in NATO countries for treatment of nausea and vomiting in cancer patients, and are used in regular medical practice throughout the world. Kytril® is administered as a single oral tablet taken once a day. Zofran® is administered as a single oral tablet taken twice a day.

On the basis of its evaluation, PG-29 concluded that both Kytril® and Zofran® meet the NATO Staff Requirement (NSR) for the intended NATO use; although Kytril® offers several advantages over Zofran®. First, Kytril® is judged to have a better technical profile. Second, Kytril® provides a clear operational advantage because it requires only one administration per day.

PG-29 recommended that Kytril® be approved for use by NATO service personnel as a self-administered means to prevent the nausea and vomiting caused by operational exposure to ionizing radiation. PG-29 drafted two NATO Standardization Agreements (STANAGs) to implement this recommendation.

Introduction

In 1979, within the framework of the Phased Armaments Programming System (PAPS) process devised by the Conference of National Armaments Directors (CNAD), the Group of Experts on Chemoprophylaxis of Radiation-Induced Injury in Man (GEC) of Panel VII on NBC Defense, wrote a Mission Need Document (MND) that identified the need to provide military personnel with a drug for the abolition or significant reduction of radiation-induced nausea and vomiting in the acute phase. Subsequently, an Outline NATO Staff Target

(ONST) was followed by the writing of the NATO Staff Target (NST) entitled *Drugs for the Prevention and Treatment of Radiation-Induced Nausea and Vomiting*. It was endorsed by the NAAG, which assigned it as PAPS Project 7.05. A statement of work (SOW) for detailed feasibility and selection studies was developed and carried out. In 1988 the Feasibility Pre-Selection Study Report on PAPS Project 7.05 was published. This report indicated that there was a new class of antiemetic compounds emerging that offered the chance of meeting the NST. Pharmaceutical manufacturers and scientists from academia and the government sector had demonstrated that $5\text{-}HT_3$ receptor antagonists are highly effective against radiation-induced nausea and vomiting and do not produce the overt, militarily unacceptable side effects associated with all previous classes of antiemetic drugs.

In 1989 the recommendations of the Feasibility Pre-Selection Study Report concerning formation of a Project Group were accepted by Panel VII and the NAAG. Thus, PG-29 was formed in October 1989 for the purpose of selecting, by mid-1996, a $5\text{-}HT_3$ receptor antagonist antiemetic drug for NATO operational use in the prevention of radiation-induced nausea and vomiting. The original membership of PG-29 comprised scientific and military representatives from Canada, Spain, the United Kingdom, and the United States. France joined in January 1991. Spain withdrew in January 1994. The final membership responsible for the conclusions and recommendations presented in the PG-29 Final Report comprised Canada, France, the United Kingdom, and the United States.

PG-29 compiled the military requirements for a $5\text{-}HT_3$ receptor antagonist antiemetic drug and developed the *NATO Staff Requirement (NSR) for a Drug for the Prevention and Treatment of Radiation-Induced Nausea and Vomiting*, based on information provided by NATO member countries. It then developed the coordinated drug testing and evaluation program defined in the PG-29 SOW. Because of the extensive research and development efforts previously carried out by civilian organizations, PG-29 was able to limit its drug testing and evaluation program to the assessment of military

operational suitability. The program as it progressed was modified in response to the results obtained from completed SOW tasks and from information obtained from other sources.

To enable completion of the testing and evaluation program within the designated lifetime of the Project Group (1989–1996), the PG-29 SOW defined a candidate drug as a 5-HT$_3$ receptor antagonist antiemetic drug that, as of January 1992, had: (a) been approved for civilian use as an antiemetic in one or more PG-29 member nations; and (b) had published data showing efficacy in humans against radiation-induced nausea and vomiting. Only two drugs were found which met these criteria: Kytril® (Granisetron; SmithKline Beecham Pharmaceuticals) and Zofran® (Ondansetron; Glaxo Wellcome, Inc.).

Both Kytril® and Zofran® were subjected to the testing and evaluation program defined in the PG-29 SOW. Literature reviews, evaluations by subject-matter experts, animal studies, and an extensive program of human volunteer studies were conducted in the four PG-29 nations to assess suitability for NATO operational use. In addition, toward the end of the project an international scientific symposium was held to ensure that PG-29 had a comprehensive understanding of the most up-to-date information on the candidate antiemetic drugs. Each PG-29-sponsored study was funded by one or more of the PG-29 nations. PG-29 met at least twice a year to review progress on tasks performed in support of the SOW, analyze the results of completed tasks, and direct future work.

The PG-29 testing and evaluation program was carried out with the cooperation of SmithKline Beecham and Glaxo Wellcome, Inc., who provided commercial-in-confidence information and supplies of the candidate drugs for study. At the request of PG-29, representatives from SmithKline Beecham and Glaxo Wellcome, Inc., provided formal presentations, participated in informal discussions, and responded to written queries.

Animal studies conducted in support of PG-29 were subject to all applicable national and local regulations. Human studies conducted in support of PG-29 were subject to all applicable national and local regulations, formal independent ethical review, appropriately obtained informed consent, and the requirements specified in the *Declaration of Helsinki.*

Military Concept of Use

Nausea and vomiting are the primary causes of performance degradation in the hours following acute radiation exposure. Although highly contingent upon the individual scenario, a large number of otherwise uninjured service personnel will become performance degraded or incapacitated due solely to emesis caused by sublethal radiation doses received in military operations. The incapacitation caused by even a single emetic episode occurring during the performance of critical military tasks is unacceptable in this context. A drug which prevents the onset of emesis will therefore contribute to mission success, preserve force effectiveness, and reduce casualties and deaths which could result from poor performance during hazardous, fast-moving military operations.

To be acceptable as an individual equipment item for operational issue, the drug should be orally self-administered, free of militarily significant side effects, and safe. It is envisaged that the drug be used in two ways:

a. *Prophylaxis.* This is the self-administration of the antiemetic drug, prior to radiation exposure to prevent the onset of nausea and vomiting.

b. *Postexposure Pretreatment.* This is the self-administration of the antiemetic drug after exposure to radiation but prior to the onset of nausea and vomiting.

Prophylaxis is the preferred operational concept because, in the absence of reliable personal dosimetry or means of predicting when exposure will occur, and taking into account the small but inevitable delay in the onset of effectiveness occasioned by oral administration, prophylaxis is the only means by which reliable and predictable protection can be achieved.

Drug Performance Factors and Evaluation Criteria

The following drug performance factors were selected by PG-29 as most critical for selection of an antiemetic drug for use by NATO service personnel as a self-administered prophylaxis and as a postexposure pretreatment.

Antiemetic Efficacy. Antiemetic efficacy is the criterion that was judged to be of greatest importance to PG-29. It is important that the selected drug, when administered as an oral prophylaxis or postexposure pretreatment, reduce significantly the proportion of individuals who experience any emesis from exposure to radiation. For those individuals in whom total protection cannot be achieved, it is important that the selected drug delay significantly the onset of the first emetic episode. Because prophylactic administration of an antiemetic drug could extend for periods of up to 2 weeks, it is necessary that the selected drug remain effective over time with repeated administration.

Absence of Militarily Unacceptable Side Effects. It is important that the selected antiemetic drug not degrade the performance of service personnel engaged in physically and/or psychologically demanding military tasks when administered as an oral prophylaxis or postexposure pretreatment.

Low Toxicity. The selected antiemetic drug should have no acute or delayed toxicity that jeopardizes the health of service personnel or their future offspring. The selected antiemetic drug should have no abuse potential.

Acceptability of Oral Dosing Regimen. It is necessary that the selected antiemetic drug be administered as infrequently as possible, consistent with maintenance of maximum protection.

Compatibility with Other Drugs. It is important that the selected antiemetic drug not alter the safety or efficacy of other drugs, or have its own safety or efficacy altered by other drugs administered to service personnel.

Regulatory Status. It is desirable that the selected antiemetic drug be approved by the civilian regulatory authorities of each NATO nation as an oral prophylaxis/treatment for radiation-induced nausea and vomiting.

Additional consideration was given by PG-29 to the factors of chemical stability and storage. It is necessary that the oral formulation of the selected antiemetic drug have a shelf life of at least 36 months at temperatures ranging from 0–50°C.

Only the antiemetic efficacy will be discussed in detail. The reader is referred to DSWA TR-96-57 and the PG-29 Final Report to the NATO Army Armaments Group for further details [1,2].

Antiemetic Efficacy

To gain a comprehensive understanding of the efficacy of the two candidate antiemetic drugs in the prevention of radiation-induced nausea and vomiting, PG-29 considered the results of all existing studies[1] in which an antiemetic drug was administered to cancer patients undergoing total body irradiation (TBI), hemibody irradiation, or localized radiotherapy, and to animals subjected to gamma or X-radiation. Also considered, in some instances, were existing studies in which an antiemetic drug was administered to cancer patients or animals treated with cytotoxic chemotherapy drugs; since it is widely believed that these drugs cause nausea and vomiting via physiological mechanisms similar to those for radiation. To gain a better understanding of how effective the candidate antiemetic drugs would be under conditions most similar to those of an operational radiation exposure, the PG-29 testing and evaluation program included animal studies in which mixed neutron-gamma radiation fields were employed.

It is important to recognize that the human efficacy data available to PG-29 were gathered from cancer patients. Many patients' underlying disease could have potentiated the vomiting response to an emetic stimulus such as ionizing radiation. In addition, the

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[1]Studies already performed by the drug manufacturers or researchers from academia or the government sector.

doses of radiation and the numbers of radiation fractions experienced by these patients is at the upper limit of the operational doses which the NSR requires the Project Group to address. Therefore, the results used by PG-29 to predict antiemetic efficacy in military personnel represent worst-case scenarios. The antiemetic efficacy of the candidate drugs can be expected to be at least as good in a healthy military population exposed to ionizing radiation during military operations.

Literature reviews conducted under PG-29 sponsorship revealed that both Kytril® and Zofran® are effective at reducing significantly the percentage of cancer patients who experience nausea and vomiting after exposure to total body radiation, hemibody radiation, localized radiotherapy, or cytotoxic chemotherapy drugs. The antiemetic drugs are most effective at preventing the severe nausea and vomiting that occur in the first 24 hours following exposure.

PG-29-sponsored studies have shown that a greater percentage of animals vomit after exposure to mixed neutron-gamma radiation than after exposure to an equivalent dose of gamma radiation and that both Kytril® and Zofran® are effective against mixed neutron-gamma radiation when administered orally in nonhuman primates. Optimal effectiveness is achieved when prophylaxis is followed up with postexposure pretreatment.

Studies conducted in humans and animals exposed to radiation or cytotoxic chemotherapy drugs have shown that the average onset time to the first emetic episode in patients or animals who do not experience total protection from an antiemetic drug is significantly longer than in patients or animals who receive no antiemetic drug. In clinical trials examining radiation-induced emesis, the percentage of patients who derive no benefit from Kytril® ranges from 0–11%, whereas the percentage of patients who derive no benefit from Zofran® ranges from 0–30%.

There are no human studies in which an antiemetic drug has been administered repeatedly for up to 2 weeks before radiation exposure. However, studies conducted in animals have shown that oral

Kytril® remains effective at preventing emesis when it is administered for up to 10 days prior to radiation exposure. There are no comparable studies with Zofran®.

Conclusions of the Project Group

The conclusions appearing below represent the opinion of the Project Group after considering all relevant data, including the commercial-in-confidence data provided by the pharmaceutical manufacturers. The combined professional expertise of the current PG-29 national representatives is such that biomedical, human performance, and operational issues could be addressed during formulation of the conclusions.

Both Kytril® and Zofran® have demonstrable efficacy in the prevention of nausea and vomiting in patient populations having various states of pre-existing ill health and exposed to relatively large doses of therapeutic radiation. Complete protection against emesis in the first 24 hours after irradiation has been achieved on average in approximately 70% of such patients. PG-29 anticipates that in a fit and youthful service population, otherwise surviving uninjured by nuclear detonation, these drugs will achieve even greater protection against expected levels of radiation exposure. Moreover, most of those in whom emesis has not been completely abolished will experience a significant reduction in the number of emetic episodes and a beneficial delay in the onset of nausea and vomiting.

Neither Kytril® nor Zofran® interferes with the performance of military tasks. Although it was not possible to conduct performance studies with the candidate antiemetic drugs under every set of climatic conditions that might be encountered by NATO forces, the absence of any adverse effects in the worst-case hot-wet environment suggests that there would be no adverse effects in other environments.

After an exhaustive consideration, and based on the combined biomedical expertise of PG-29 and its consultants, the Project Group concluded that there

is no likelihood that Kytril® or Zofran® will cause immediate or delayed toxicity, cancer, or cardiac dysfunction in healthy service personnel when the drugs are administered according to the recommended oral dosing regimen. In addition, neither Kytril® nor Zofran® has been shown to have developmental toxicity in established animal models. However, the pharmaceutical manufacturers do not recommend that these drugs be administered to pregnant women.

The Project Group concluded that the optimal prophylactic dosing regimen for the two candidate drugs for NATO operational use are:

- Kytril®: 2 mg administered orally once a day.

- Zofran®: 8 mg administered orally twice a day.

PG-29 has concluded that NATO service personnel would benefit from re-administration of the antiemetic drug as soon as possible after an acute operational radiation exposure has occurred, regardless of the time interval that has elapsed since administration of the last prophylactic dose.

Having found no substantiated adverse drug-drug interactions between Kytril® or Zofran® and other drugs, PG-29 concluded that they are compatible with the other medications used in service personnel.

The number of NATO countries in which there is civilian regulatory approval for Kytril® or Zofran® for prevention of chemotherapy or radiation-induced nausea and vomiting is sufficient to conclude that these drugs are widely considered safe and effective.

From the data available to date, PG-29 concluded that both Kytril® and Zofran® are able to meet military stability and storage requirements.

On the basis of the completed drug testing and evaluation program that addressed efficacy, safety, side effects, and maintenance of combat capability and command reliability, PG-29 concluded that both Kytril® and Zofran® meet the *NATO Staff*

Requirement for a Drug for the Prevention and Treatment of Radiation-Induced Nausea and Vomiting.

For the intended NATO use, PG-29 recommends that Kytril® be approved for use by NATO service personnel as a self-administered means to prevent the nausea and vomiting caused by operational exposure to ionizing radiation and suggests the following drug administration schedule:

a. Kytril® should be taken prophylactically, on command, as a 2-mg tablet by mouth (p.o.), once daily. Repeated daily prophylaxis may continue for the 14 days required by the NSR.

b. One 2-mg tablet of Kytril® should be taken as soon as possible after an acute operational radiation exposure has occurred, regardless of the time interval that has elapsed since the administration of the last prophylactic dose, if any. Subsequent to radiation exposure, the drug should continue to be taken according to the above schedule for each postirradiation day until otherwise advised by command or medical authority.

References

Documentation supporting the conclusions of PG-29 can be found in the following:

1. *Final Report, Selection of a Drug for Prevention of Radiation-Induced Nausea and Vomiting,* Contract DNA001-93-C-0204, published as DSWA TR-96-57.

2. *NATO Project Group Final Report.* AC/225-D/1386, AC/225(PG.29)D/28.

Review of Potential Biomarkers of Radiation Exposure

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Introduction

Maintaining operational capability in a nuclear conflict will require knowledge of the levels of radiation military personnel have received and timely decisions concerning combat readiness and the triage of irradiated personnel.

Currently, physical dosimetric methods are the primary means used to assess exposure levels. The initial symptoms of radiation injury are used to determine combat readiness. Echelon II support provides the capability for lymphocyte counting to categorize acute radiation damage in the 100–500 cGy range.

However, personnel dosimeters are limited by their particular location and specific radiation response. On the individual level, human response varies considerably depending on the nature of the exposure and a variety of other factors. The suitability of a particular biological dosimeter can be assessed by reference to the list of basic requirements (see Box: Biodosimetry Requirements). It is important to develop simple, fast, reliable biological indicators of radiation damage. To be realistic, such a biological dosimeter must be capable of responding predictably to doses ranging from 100–500 cGy. It must be sufficiently simple to be used in forward field situations involving limited (echelons 0, I, and II) medical facilities, and must be capable of providing a rapid result for a large number of individuals. An instrument-based automatable technology using readily available biological samples, involving minimal handling, and a direct, unambiguous analysis with little or no interpretation or manipulation, is clearly preferred [1,2].

Biodosimetry Requirements

- Sensitive
- Reproducible
- Specific
- Stable
- Non-invasive
- Dose-dependent
- Direct
- Simple, inexpensive
- Fast, portable
- Low intra- and inter-individual variability
- **Physical dose** (independent of dose-rate, LET, radiation type or exposure history) or
- **Biological dose** (a measure of individual radio-sensitivity or relative risk)

Several competing technologies have been critically compared (table 2) and their strengths and weaknesses discussed [3]. These include two classes of biological dosimeter. The biophysical type (e.g., ESR) operates like a physical dosimeter

Table 2. A survey of biological indicators.

Technique	Assay	Sample	Sensitivity (Gy)
Hematological	Electrophoretic mobility, membrane permeability mitotic index, cell loss and recovery	Lymphocytes	2–5
Immuno-fluorescence	Cell-surface markers	Lymphocytes	0.1
Cytological	Micronuclei, chromosomal aberrations	Lymphocytes	0.1
Genetic	Mutations, base damage	Lymphocytes	0.1
Biochemical	Small molecule release or loss	Blood, urine	0.5
Biophysical	ESR	Bone, hair, nails, teeth	1.0

in quantitating a change in a biological sample that is a direct measure of absorbed dose, independent of dose-rate, radiation quality, and other confounding factors (e.g., age, lifestyle, health, synergistic effects of heat, etc.). The other type of biological dosimeter estimates changes in biological samples (e.g., biochemical, immunological, cytological, genetic) that reflect individual radiosensitivities, and is really a measure of biologically-relevant dose. These dosimeters are indicators of stress response; the body reacts to radiation in ways to maximize the benefit/risk ratio [4]. The body's constitutive or inducible defences include protection against initial free radical-mediated oxidative damage, repair of potential DNA damage, immunological (NK cells) and genetic processes (apoptosis) to control and eliminate irreversible damage. Many of these defence mechanisms are subject to surveillance and signalling feedback networks, and are triggered by low levels of stress as part of the overall adaptive response.

Criteria for Developing New Strategies

Ideally, biotechniques applicable to acute radiation dose monitoring should be based on a firm mechanistic foundation at the cellular and molecular levels in quantitating the biological consequences of radiation exposure. In human radiobiology, the hematopoietic system is the most radiosensitive, and the lymphocyte which remains predominantly in the non-proliferative G_0 or interphase state, is the most important biological sample for biodosimetric purposes. In the white-blood cell, as with other cells in the body, it is radiation damage to the genetic material (the DNA) that is the most crucial; this is reflected in the emphasis on genetic and cytogenetic markers as important potential biodosimeters. In order for a cellular or molecular assay to fulfill the requirements for a suitable biological dosimeter, it must be amenable to automation, preferably as a direct readout device utilizing a readily-available sample and a simple sample-handling procedure. In addition, any prospective biodosimeter should have adequate reliability, reproducibility, radiation specificity, cost-effectiveness, short-duration and high through-put [3].

Biophysical Dosimeters

The human body is the ultimate biodosimeter. When humans are irradiated, free radicals are produced and remain trapped in solid materials such as tooth, bone, hair, and nail. In these materials, the number of free radicals trapped is directly proportional to the absorbed dose received [5]. This form of biological marker is analogous to a conventional physical dosimeter in that it is not affected by biological responses to ionizing radiation such as DNA repair and adaptation. Consequently, the biophysical dosimeter, unlike other forms of biological dosimeters, is independent of the nature of the radiation exposure (radiation quality, LET, fractionation, mixed fields, dose-rate, etc.), or the individual's radiosensitivity.

Electron Spin Resonance (ESR). The energy levels of any paramagnetic species containing an unpaired electron, which includes free radicals, are divided into a higher and lower state in the presence of a magnetic field. By applying microwave power to a sample in a wave-guide cavity which is experiencing a continuously changing magnetic field, a magnetic resonance spectrum is obtained when the unpaired electrons are promoted to their higher energy states. The intensity of such an ESR spectrum is a direct measure of the number of free radical "spins," which in turn is directly proportional to the absorbed dose.

In vitro studies have been carried out with human hair, tooth, finger nail, and bone samples [6–9]. Desrosiers [10] estimated doses received by a radiation worker involved in an accident at a Co-60 radiation processing plant by using a bone sample from the victim's amputated leg. Recent developments are being directed at *in vivo* dosimetry of tooth enamel [11].

Biological Dosimetry

A wide variety of techniques is under investigation, but no one technique is capable of satisfying the present requirements [12–14]. The following biological indicators utilize biochemical, immunological, or genetic changes occurring as a consequence

of radiation exposure. These types of damage reflect the overall biological response of individuals to radiation and other forms of oxidative stress.

Hematological Indicators. The extent and rate of suppression of lymphocyte counts are the only direct and rapid methods of estimating acute exposures in the 100–500 cGy dose range [15]. Typically, an individual's lymphocyte count is reduced ~50% following a dose of 100–200 cGy, and the depletion time is ~ 1–2 days. Higher doses produce proportional decreases in lymphocyte counts and concomitant depletion times.

Immunological Indicators. The immune system, as well as being a potential bioindicator [16,17], plays an important role in our defense against a hostile environment, serving as a surveillance and signalling system, as well as a feedback mechanism to prevent incursion of foreign material and to control aberrant biological processes and disease states. It maintains a high level of fidelity among the cells of the body, and is an important component of the stress response. A wide range of sensitivities exists between individual immune systems. As with chemicals and other environmental agents, some individuals are hypersensitive to ionizing radiation. Whether this is beneficial or harmful is still open to question.

Work done at AECL [18] has shown that low doses of ionizing radiation lead to the over-expression of IL-2 receptors on lymphocyte cell surfaces. This immuno-stimulation response is highly sensitive to radiation. However, at intermediate dose levels (1–20 cGy), the response plateaus and is not dose-dependent, rendering this assay unsuitable as a biological dosimeter. Likewise, the radiation-induced destruction of surface receptors [19] levels off for various lymphocyte sub-types (T-, B-, and NK-cells) after an initial dose response at low doses (1–20 cGy). This non-linearity of the immune response at high acute exposures may be a result of the preferential destruction or disruption of the most radiosensitive hemopoietic stem cells, leaving a depleted population of less responsive, more radioresistant survivors.

Cytological Indicators. Cytological analysis of genetic damage to peripheral blood lymphocytes [20,14] is the preferred method of assessing radiation dose in the case of accidental exposure. The most common end-points are chromosome aberrations, including deletions and translocations [21]. More recently, the need to improve quantitative analysis has prompted considerable research towards the development of the micronucleus [22] and apoptosis (programmed cell death) [23] assays.

Because lymphocytes from peripheral blood are in a G_0 resting phase (interphase), it is necessary to stimulate them to divide by using a mitogen such as phytohemagglutinin (PHA), and to maintain them in cell culture for up to 48 hours prior to scoring genetic damage. In the case of chromosome aberrations, dividing cells are treated with a mitotic inhibitor colcemid (or colchicine) to arrest them in metaphase allowing whole chromosomes to be visualized in a metaphase spread. Because of the labor-intensive, subjective nature of scoring the complex chromosome aberrations, attempts have been made to simplify and automate this assay. One recent development, Fluorescence *In Situ* Hybridization (FISH), utilizes fluorescently-labelled DNA synthetic probes introduced into irradiated cell nuclei to "paint" chromosomes and regions containing complementary sequences. Specific damage in these painted chromosomes is highlighted and easily scored using a fluorescence microscope. New advances in digital image analysis are helping to automate the scoring of chromosome damage using FISH technology [24,25].

A simpler assay for damage in genetic packaging and processing employs a blocking agent, cytochalasin B, that inhibits cell division after mitosis, converting every viable cell into a binucleate cell. Any radiation damage that affects the cell's ability to faithfully replicate its genetic material results in the formation of extra-nuclear DNA pieces or micronuclei which are easily counted either manually or by using digital image processing of the microscopic image—produced by staining with a fluorescent DNA-intercalating probe.

Another self-induced process of DNA degradation occurs during apoptosis when a cell undergoes self-directed suicide to avoid passing on potentially harmful genetic information to future generations. The characteristic self-destructive disassembly or "laddering" of the genetic material can be quantitated using polyacrylamide- or single cell-gel electrophoresis, flow cytometry, and immunochemical fluorescence analyses [23,26].

DNA Organizational Damage. A consequence of radiation-induced DNA damage at the molecular level is the introduction of conformational and topological changes into the genomic architecture which may have serious biological consequences. Numerous sub-cellular assays have been developed (table 3) to study this level of genetic damage resulting in a disorganization of the nuclear chromatin. They all have the advantage of simple, rapid, direct analysis, based on a variety of analytical techniques requiring minimal sample handling and subjectivity. Techniques involving single-cell analysis include the HALO [27], nucleoid [28] and COMET [29,23] assays, in which lymphocytes from irradiated individuals are subject to chemical treatments (detergent, alkali, high salt, oxidant) aimed at lysing the

Table 3. DNA organization damage assays.

Damage	Method/Parameter	Detection
Breaks	Size, shape, MW	Ultracentrifugation, light scatter
Denaturation, melting	Density, double-strandedness	Viscosity, sedimentation, hyperchromism, alkaline elution
	FADU	Fluorescence
Conformation, topology	FLI	Time-resolved fluorescence
Charge density, polarity	Polarography, ionography	Electrochemical, partitioning, conductivity
Aggregation	COMET HALO, nucleoids, apoptosis	Electrophoresis, fluorescence microscopy, flow cytrometry
Fragmentation	Low MW product analysis; site-specific damage; metabolites	HPLC, chemical, biochemical, immunochemical tests

outer membrane and relaxing the nuclear material. Most recent developments in assaying such DNA damage utilize changes in size and shape, mobility, diffusion, and charge density. Detection and visualization involve staining with an intercalating agent and viewing in a fluorescence microscope. Halos are produced from radiation-induced disaggregation of the nuclear material resulting in an increased light scattering around the periphery of the nucleoids. When these partially-lysed nucleoids are run in an agarose gel electrophoresis unit, the low molecular weight nuclear material streams out of the nucleus forming a comet-like pattern in which the tail distribution and elongation are dose-dependent.

Single- and double-strand breaks can be specifically scored by alkaline or neutral elution techniques [30]. The results of these assays are strongly influenced by DNA repair processes, making the methods of collecting, storing and processing the sample, and particularly the timing of the steps with respect to prior irradiations, very important in terms of reproducibility and obtaining a quantitative analysis. Another method to score specific DNA damage, including site-specific damage, is to use an immunoassay in which antibodies to particular DNA damage, such as single strand breaks or altered nuceotide damage [31], are reacted with their specific antigenic substrate, and the antibody-antigen complex tagged with either a colorimetric (ELISA) of fluorimetric (FIA) probe.

Biochemical Indicators. The release of certain biochemicals into various body fluids has the potential to serve as a simple, rapid method of indicating the overall metabolic consequences of whole body exposure [32]. Some examples include serum albumin adducts, thymidine products and various enzymes including amylase and diamine oxidase, GSH adducts, and specific nucleotide and protein metabolites in urine [33], and hemoglobin adducts in peripheral blood.

Future Developments

Clearly, what is needed for a forward field application is a straightforward, direct, automated technique requiring minimal sample handling and

manipulation, utilizing a non-invasive biological sample, capable of monitoring an individual response to single or multiple acute radiation exposures. Of the techniques discussed in this review, ESR and other forms of analytical spectrometry (OSL, FTIR, GC-MS), and instrument-based cytological techniques offer the most promise [2]. Other methods requiring new technology or combinations of existing technologies (FISH, cytometry, image analysis, chromatography, immunoassay, biosensors) also appear to have considerable potential (table 4). These may include the use of solid-state probes to monitor, non-invasively, one or a series of low molecular weight products or metabolites in body fluids, including blood, urine, sweat, saliva and tears. With the advent of digital imaging, any data obtained by flow cytometry, phase-contrast, or fluorescence microscopy are potentially automatable and may provide the basis for future direct, large-scale cytogenetic screening. Utilizing the body's immune system to pin-point and score minute quantities of specific products, and using multiplexing to develop patterns of products synonymous with a radiation signature may be a future option.

Outstanding Issues Requiring Attention

Once a satisfactory technology is developed, a number of QA issues remain to be resolved. These include the problems of biological variability, radiation specificity, dose-effect relationships, and confounding variables. However, these can be dealt with once the appropriate technology is developed simply by the application of due diligence in terms of data collection, analysis and evaluation, possibly involving National and International inter-comparisons and the adoption of standardized protocols.

Conclusion

Biological dosimeters measure the biologically relevant effects of radiation on exposed individuals as an estimate of effective dose; whereas biophysical dosimeters are analogous to personal dosimeters

Table 4. Promising technologies.

Fast, simple, instrument-based, non-subjective, minimal sample handling, capable of automation, validation, and calibration	
Biosensors	Non-invasive, solid-state probes of low MW products in body fluids
Immunoassays	Highly specific, selective antibodies against radiolytic products or metabolites; ELISA, FIA, TRIFA
Biophysics	Highly sensitive, analytical spectroscopic techniques; AFM, MS, OSL
Biotech	Differential display (multiple gene expression); mutation spectral analysis (radiation specificity); DNA probes/FISH, PCR, and RFLP; adaptive response; stress response

in providing a measure of absorbed dose. The biological and biophysical dosimeters, possibly in combination, may provide an important role in the assessment of acute radiation exposures in the event of a nuclear strike or accident and the subsequent clean-up operations. Most of the techniques are applicable to the detection of relatively large radiation exposures at relatively short times after exposure. However, no one technique satisfies all the necessary requirements; and each technique is subject to considerable uncertainty in terms of biological and other variables. The development of specific and reproducible tests, and their inter-comparison, will help resolve some of the uncertainties and will lead to a greater understanding of the underlying mechanisms of radiation-induced injuries and of appropriate intervention strategies to alleviate them.

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Appendix B

Session II

Alterations of Hematological Parameters by Radiation

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The changes produced by radiation exposure to the blood forming tissues were recognized early in this century. The huge body of related publications that have since accumulated have been the subject of a number of reviews [e.g., 1–9]. The objective of this paper is to consider only those alterations whose detection methods lend themselves to practical application in the military-field environment within relevent time constraints. Their use should facilitate accomplishment of the mission by helping decision makers to evaluate the potential functional competence of the radiation-exposed personnel in the hours, days, or weeks following irradiation. An additional objective is to provide guidance concerning the medical evacuation of irradiated personnel. The information presented also may help in planning the subsequent treatment of radiation casualties, or may even help estimate the physical dose absorbed; however, the primary focus of the initial diagnostic tests remains the assessment of the severity of injury in the individuals being examined.

The diagnostic problems imposed by the prophylactic use of antiemetics to block the nausea and vomiting, two useful early indicators of radiation overexposure, and by the limitations in the performance of many laboratory tests in a field environment, are evident when one considers the diagnostic parameters

of a typical triage scheme for the management of radiation accidents, shown in figure 1 [10,11]. This triage scheme for clinical diagnostic and prognostic classification according to severity of injury was proposed utilizing the time of appearance and severity of the prodromal signs and symptoms, and of the alterations in the early blood-cell counts. Thus, the difficulty is increased to define the fastest and simplest reliable tests to generate the necessary triage information quickly. It should be borne in mind, however, that the accuracy and sensitivity of the initial diagnostic procedures need not be greater than that required to make the triage decisions.

Simplicity would dictate the use of a single hematologic indicator showing the extent of radiation injury and resultant impairment for radiation exposure in a military setting. Unfortunately, shortcomings become evident with each hematologic parameter when used as the sole prognostic indicator. For example, the radiosensitivity of the hematological precursor cells would seem to make marrow examination a logical diagnostic approach; however, technical performance difficulties in the field, geographic variations at different aspiration sites secondary to the usual non-uniformity of exposure, asynchrony between marrow and peripheral blood changes over time, and psychological problems associated with intensive investigation during a relatively asymptomatic period, make this unfeasible on a repetitive basis at lower than echelon III care.

Peripheral blood counts may be feasible with field-hardened automated equipment at echelon II or III facilities. Unfortunately, each cell line used alone presents some interpretive difficulties. For example, the particular radiosensitivity of the lymphocyte line leads to prompt lymphopenia, making this a helpful

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│ 1. Observe and record time of onset of clinical signs and symptoms. │
│ 2. Perform daily blood count.                           │
└─────────────────────────────────────────────────────────┘
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        ┌──────────────────────────────┐        ┌──────────────┐
        │ Nausea, Vomiting, Diarrhea   │   +    │ Injury Group │
        │      within minutes          │───────▶│      V       │
        │            and               │        └──────────────┘
        │ Ataxia, Disorientation, Shock, Coma │
        │     in minutes to hours      │
        └──────────────────────────────┘
                    │ −
                    ▼
            ┌──────────────────┐
            │  Injury Groups   │
            │   I, II, III, IV │
            └──────────────────┘
                    │
  ┌──────────────┐  − ┌──────────────────────┐
  │ Injury Group │◀───│  Nausea and/or Vomiting │
  │      I       │    │  and some derangement of │
  └──────────────┘    │  blood count within 2 days │
                      └──────────────────────┘
                            │ +
                            ▼
                    ┌──────────────┐
                    │ Injury Groups │
                    │   II, III, IV │
                    └──────────────┘
                            │
  ┌──────────────┐  − ┌──────────────────────┐
  │ Injury Group │◀───│ Marked leucocyte and lymphocyte │
  │      II      │    │  count derangement in 3 days │
  └──────────────┘    └──────────────────────┘
                            │ +
                            ▼
                    ┌──────────────┐
                    │ Injury Groups │
                    │    III, IV    │
                    └──────────────┘
                            │
  ┌──────────────┐  − ┌──────────────────────┐  + ┌──────────────┐
  │ Injury Group │◀───│ Diarrhea within 4 days and │───▶│ Injury Group │
  │     III      │    │ marked platelet derangement │    │      IV      │
  └──────────────┘    │  within 6 to 9 days  │    └──────────────┘
                      └──────────────────────┘
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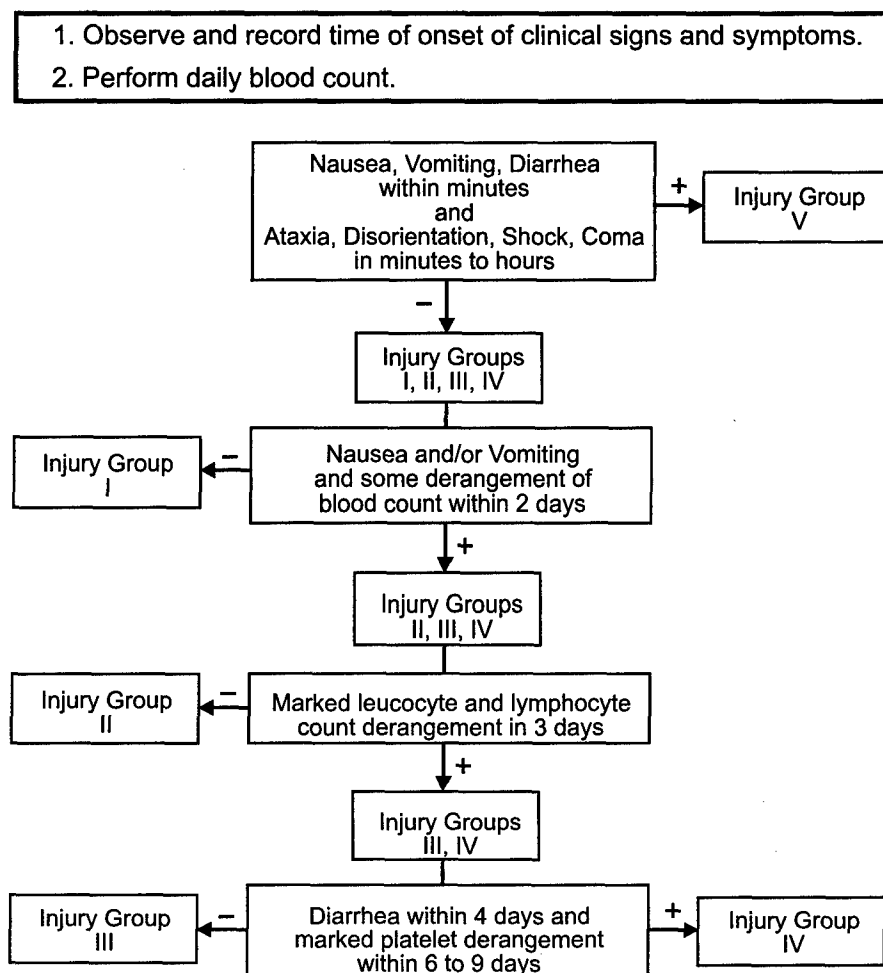
Fig. 1. Radiation triage flow chart.

early indicator; however, it may be seen in otherwise unimpaired individuals. In addition, significant sublethal injury may produce as severe and fast a lymphocyte fall as that seen in the mortally injured. Furthermore, total leucocyte and granulocyte levels may *increase* promptly due to mobilization from tissues in a nonspecific response to stress before the deficit in production supervenes.

The injury classification approach that Thoma and I developed many years ago for radiation accident management [10], using the relatively quick and simple blood-cell count, could enhance the yield of available prognostic information. We studied the published clinical data related to the small population of accidentally over-exposed radiation workers who were generally in good health at the time of exposure and were closely clinically observed.

Based on an analysis of clinically significant accidents involving 32 patients for whom there was sufficiently detailed medical information, we promulgated recommendations for the diagnosis and management of acute radiation injury. This approach, which does not require the use of physical dosimetric information that is usually difficult to obtain promptly in an accident setting, has been generally accepted and its details disseminated over the years in a variety of publications [e.g., 12,13,7,8,14]. Because of its potential utility in a military environment, it will be summarized briefly here.

Based on a detailed review of the clinical data, we classified the patients into five injury groups that are described briefly in table 1. Next we reviewed the clinical laboratory data to see which

**Table 1.** Modified Thoma-Wald (1959) injury group classifications.

	Clinical radiation injury groups	
Group	Prognosis	Clinical features
Group I	Survival assured	Generally asymptomatic or with minimal prodromal anorexia and nausea (few hours). No significant impairment.
Group II	Survival probable	Mild Acute Radiation Syndrome (ARS). Prodromal nausea, vomiting.(1-2 days). Mild hematologic abnormalities with little consequent clinical impairment for at least 2-3 weeks.
Group III	Survival possible	Classical ARS prodroma (1-2 days). Possible performance decrement from fatigue thereafter with major hematologic derangement producing life-threatening complications in 2-3 weeks and requiring major supportive therapy.
Group IV	Survival improbable	Accelerated severe ARS prodroma, including diarrhea, followed by weakness and recurrent GI problems. Major hematologic complications if survival exceeds 1-2 weeks.
Group V	Survival impossible	Immediate violent ARS prodroma with disturbances in consciousness and homeostasis leading to shock, coma and death in hours to 1-2 days.

tests gave the earliest and most accurate prognostic information. Only the hematologic observations will be considered in this presentation.

Figures 2 and 3 show typical changes for patients in Injury Groups II, III, IV, and V. It is evident that shortly following exposure, the blood changes are highly dependent on when the observations are made, with different cell lines changing asynchronously.

To compensate for the lack of a perfect single indicator and to amplify the information gained early postexposure by utilizing all of the blood count data, we developed a method to rank hematologic abnormality using the changes in all of the cell
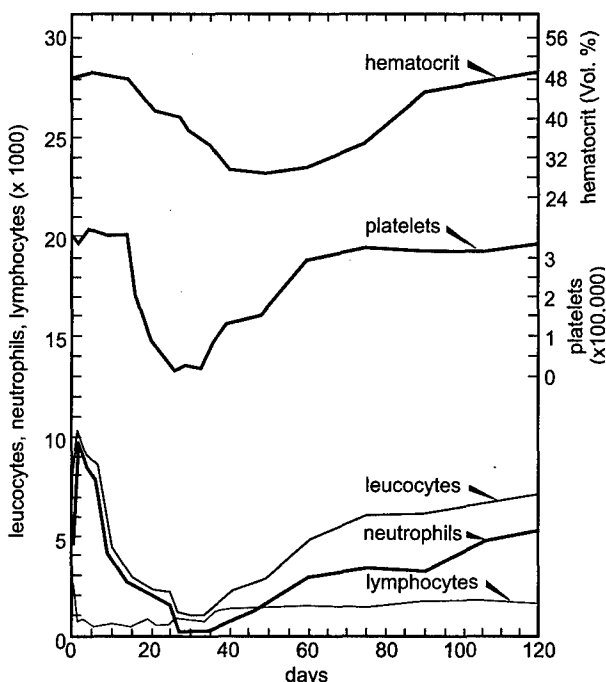
Fig. 2. Typical blood count for group II.
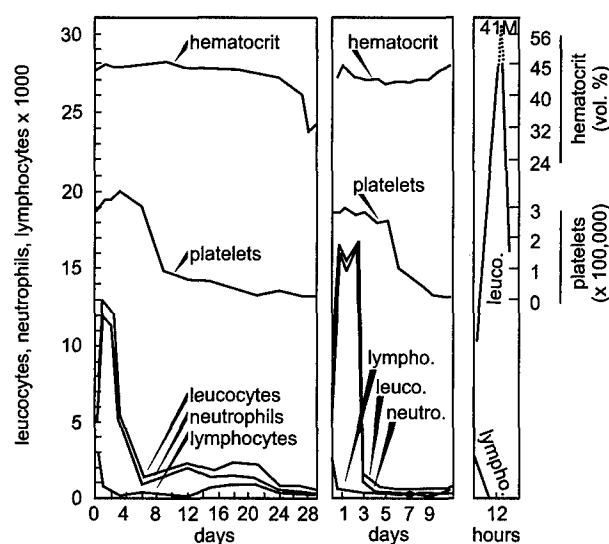
Fig. 3. Typical blood counts for groups III, IV, and V.

lines. Ranges were defined for four levels of increase or decrease from the normal range [15] for each line, as presented in table 2. The composited data, called the hematologic injury scores, were cumulated and plotted up to 60 days or until earlier death. Knowing the outcome in each case, it became evident that the rate of accumulation of evidence of hematologic injury correlated well with the severity of injury or with the injury group classification that had been made on the basis of the clinical manifestations of the radiation overexposure. There were clearly different rates of accumulation of the hematologic injury score in the individual cases and in the clinical injury group means. More details are available in the original publications [10,11] and in the other references cited above.

For this presentation, a plot of the cumulative scores of the individuals in our accident study covering the first 30 days postexposure is provided in figure 4. It reveals the rate differences even in the first few days postexposure. The fatal cases, in Injury Groups IV and V, showed the steepest slopes; but even Injury Group I patients could be distinguished from those in Groups II and III within the first 2–3 days postexposure. None of the cumulated data for any

Table 2. Rank values assigned according to magnitude of abnormality of hematologic indices.

Test	Units	Normal	Rank: 1	2	3	4	Rank: 1	2	3	4
			For increase above:				For decrease below:			
Hemoglobin	gms %	M-15.8	18	19	20	21	14	12	10	8
		F- 13.9	16	17	18	19	11.5	10	8.5	7
Erythrocytes	mill/mm^3	M- 5.4	6.0	7.0	8.0	9.0	4.5	3.5	2.5	1.5
		F- 4.8	5.5	6.5	7.5	8.5	4.0	3.0	2.0	1.0
Hematocrit	vol. %	M-47	54	56	58	60	40	35	30	25
		F- 42	47	49	51	53	37	32	27	22
Leucocytes	1000/mm^3	7.4	12	18	24	30	4.0	3.0	2.0	1.0
Neutrophils	1000/mm^3	4.4	7.7	14	21	28.0	1.8	1.3	0.9	0.5
Lymphocytes	1000/mm^3	2.5	4.8	7.0	10	12.0	1.0	0.75	0.5	0.3
Platelets	1000/mm^3									
Rees-Ecker		405	545	700	850	1000	273	200	100	30
Brecher-Cronkite		257	440	600	750	900	140	100	50	30
Fonio		234	350	500	650	800	130	100	50	30
Dameshek		716	900	1000	1500	2000	500	350	100	30
Reticulocyte	% RBE	1.5	4	8	15	25	0.5	0		
Erythrocyte	mm/hr	M-5	10	20	30	40				
Sediment rate		F-10	20	30	40	50				

*Expressed as "universal mean" value - taken from Albritton (1952).
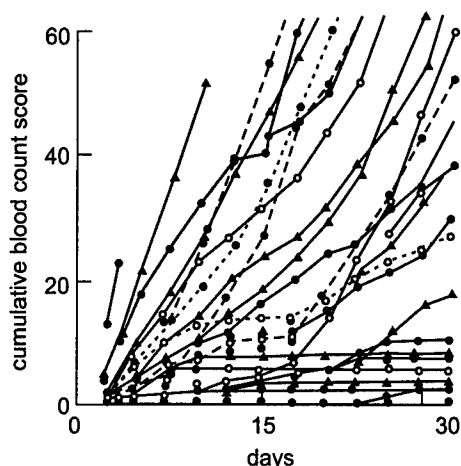Table modified from Wald et al. (1961).

Fig. 4. Radiation accident patients: cumulative blood count scores.

single cell line's series of measurements gave as great a response as the composited injury score within the very early postexposure time period of interest. The rate of change between two or more early composite data points could be determined quickly with a computerized slope analysis and the injury group prognosticated. Therein lies the potential applicability of this approach to the triage of the antiemetic-protected personnel. If blood counting, preferably automated, is feasible at echelon II or III, this methodology, used in conjunction with other clinical indicators, could be worthy of consideration to facilitate early triage, operational planning, and evacuation decisions.

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Prediction of Clinical Course Through Serial Determinations

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To perform an efficient triage for radiation exposure injuries it will be necessary to define the categories of radiation lesions. Using hemopoiesis as the reference system to categorize radiation effects would offer the most relevance from a clinical perspective and would allow medical personnel to anticipate the patient's further clinical course, the patient's required care level, and his performance degradation. Thus, this clinically oriented categorization will be helpful in determining the resources which will be necessary for contingency planning in terms of the required transportation, medical personnel, medical materials, and the patients' performance capability.

The entire clinical course of granulocytes, lymphocytes, and platelets has been differentiated into five categories. These five categories have been verified against acute radiation syndrome case histories from the International Computer Database for Radiation Exposure Case Histories (ICDREC) [1], based on the Moscow-Ulm Radiation Accident Database [2]. With 558 case histories from persons who suffered the consequences of acute radiation exposures from 45 accidents in 15 countries, these case histories were collected in a standardized Pre-Computer Case Report [3] containing up to 901 items characterizing the entire clinical history for the first 100 days after the accident. The categories are:

- Category 5: Exposure absolutely lethal (palliative therapy only), 3 out of 558 patients.

- Category 4: Essentially irreversible injury to stem-cell pool (stem-cell transplantation essential), 29 out of 558 patients.

- Category 3: Potentially reversible injury to stem-cell pool (cytokine therapy, extensive antibiotic therapy, platelet support), 52 out of 558 patients.

- Category 2: Moderate effect on stem-cell pool resulting in transient blood cell changes with granulocyte counts dropping several times below 0.5×10^9 per l and platelet counts dropping several times below 50×10^9 per l (84 out of 558 patients).

- Category 1: Very moderate effect on stem-cell pool resulting in blood cells never dropping below critical thresholds (granulocyte counts below 0.5×10^9 per l or platelet counts below 50×10^9 per l), 390 out of 558 patients.

What parameters serve best to predict the category of radiation lesion? The hematological parameters have proven to be most effective in predicting the course of the radiation disease. In combination they are very specific. There is little else known that causes lymphocytes to decrease, a granulocytosis, a consecutive granulocyte decrease, and a platelet decline in a certain time pattern. A good estimate of the future clinical course, applying the whole set of hematological indicators, can be obtained within 7 days after exposure. The granulocyte counts, especially between day 4 and day 7, allow for reliable clinical decision making for individual patients [4]. Blood samples taken regularly after the exposure, are prerequisites for a reliable prediction. A peripheral blood count with an additional lymphocyte count, which requires blood from the ear or finger tip, provides the necessary data. Whereas, the late onset of important signs, such as granulocytopenia and thrombocytopenia, reduces their early diagnostic efficacy.

Decision aids for early triage would be tremendously helpful following an event in which a large number persons are potentially irradiated. Triage does not mean to finally determine the patient's clinical category; rather, it offers a first hint of the severity of the lesions. The clinical category will have to be finally established by sequential diagnosis. To estimate resources (e.g., transportation, medical care facilities) early indicators will need to be sufficiently precise only to the extent of the number of casualties due to the statistical power built into the casualty number. Consequently, to assess in about 24 hours the radiation damage to personnel, additional indicators that allow for earlier

Table 3. Laboratory parameters to roughly estimate the patients' clinical category.

	Lymphocyte decline within 36 hours	Serum amylase increase within 24 hours	Reticulocyte decline within 48 hours
Category 5	~ 0.0x10^9/l	2,500 U/l	~ 0x10^9/l
Category 4	~ 0.1x10^9/l	~ 2,200 U/l	~ 5x10^9/l
Category 3	~ 0.3x10^9/l	~ 1,500 U/l	~ 10x10^9/l
Category 2	~ 0.8x10^9/l	~ 500 U/l	~ 20x10^9/l
Category 1	~ 2x10^9/l	~ 100 U/l	~ 50x10^9/l

differentiation and are correlated with the clinical categories are required.

Within the first 24 hours, in addition to the lymphocyte decrease, a combination of a significant serum amylase increase, the reticulocyte decrease, and a marked lymphopenia, will help to preliminarily establish prognosis. Table 3 shows how lymphocyte counts, serum amylase measurements, and reticulocyte counts after radiation exposure correlate with the categories described above. The numbers provide rough estimates only. The idealized curves based on the pathophysiological behavior have even greater evaluative attributes. One serum amylase measurement within 24 hours without the exact time after the radiation exposure is not meaningful. Serum amylase rises from about 100 U/l at the time of the exposure to a maximum, which can be more than 3000 U/l. The maximum lies between 12 and 24 hours depending on the biologically effective radiation dose. The serum amylase then returns to normal within 48 hours.

The specificity of the lymphocyte decline, the serum amylase increase, and the reticulocyte decline is much lower than the granulocyte decrease and the platelet decrease. Despite the fact that no major treatment decision should be made on these data, they can serve to estimate the required medical care capacity, the transportation capacity, and the number of casualties. Blood samples should be taken three times a day during the first 36 hours after the exposure, if possible, to determine the lymphocytes, serum amylase, and the reticulocytes. The high frequency of sampling is due to the dynamic in the parameters.

Taking blood from the ear or finger tip can provide the specimen to determine the parameters.

The most promising clinical parameters to predict the clinical category from the ICDREC seem to be vomiting, nausea, diarrhea, abdominal pain, enlargement of parotid glands, primary erythema of the eye, initial conjunctival hyperemia, skin erythema, mucous membrane erythema, radiation conjunctivitis, fatigue, and elevated body temperature. Only vomiting, nausea, and fatigue are sufficiently correlated to have some predictive value. The specificity is lower than for the lymphocyte counts, the serum amylase, and the reticulocyte counts. The correlation between the first occurrence of the symptom after exposure and the clinical categories is shown in table 4. If effective antiemetics are given, vomiting and nausea could lose their predictive value; and fatigue would be the only non-laboratory indicator.

How does the administration of antiemetics prior to or shortly after exposure affect the diagnosis of

Table 4. Correlation between the patients' clinical category and the primary reaction.

	Vomiting	Nausea	Fatigue
Category 5	~ 30 minutes	~ 30 minutes	~ 30 minutes
Category 4	~ 1 hour	~ 1 hour	~ 1 hour
Category 3	~ 4 hours	~ 2 hours	~ 2 hours
Category 2	~ 6 hours	~ 3 hours	~ 4 hours
Category 1	Sometimes	Sometimes	~ 6 hours

radiation lesions? The question can hardly be answered from the ICDREC since only 33 individuals received an antiemetic therapy. Assuming that antiemetics are effective against nausea and vomiting only, the above mentioned set of early diagnostic indicators will be reduced by two parameters. The ICDREC patients who received antiemetics showed no significant difference in the onset of vomiting and nausea compared to the other patients.

If available, dosimeter readings will provide another clue for establishing prognosis; although the results should be considered with extreme care due to the significant inhomogeneity of exposure. The dose estimates for patients of different clinical categories are normally distributed with different means and a high variance. The clinical categories can be statistically differentiated by the dose estimates.

Though the grading appears to be consistent and reflects dosimetry relatively well for larger sample sizes (see table 5), for individuals there is limited correlation between the dose estimates and the clinical category.

To reduce the uncertainty inherent to all biological data, frequent measurements of both signs and symptoms is of paramount importance during the first hours and days after exposure. This diagnostic approach has been named sequential diagnosis.

Conclusion

An exploratory analysis of the ICDREC data concerning the question, which early indicators can help to predict the clinical category of patients after acute

Table 5. Correlation between the patients' clinical category and the estimated dose.

	Dose
Category 5	14 Sv
Category 4	~ 11 Sv
Category 3	~ 5 Sv
Category 2	~ 3 Sv
Category 1	~ 1 Sv

irradiation, suggests that lymphocyte count, serum amylase, and the reticulocyte count are the most promising laboratory parameters. However, facilities to perform blood counts and measure serum amylase must be available. Vomiting, nausea, and fatigue are the early clinical parameters which are correlated with the clinical category. Dose estimates show a good correlation with the clinical category; but on the individual level there is limited correlation between the dose estimates and the clinical category.

These early indicators provide an initial estimate of the severity of injury which can be used to assess transportation and medical capacity needs and to estimate the number of casualties. The final clinical decision must be based on the patient's state, especially taking into account the granulocyte counts between days 4 and 7 and the platelet counts.

A diagnostic guideline should be developed to determine the extent of the radiation lesions. To determine the reliability of the guideline, it should be evaluated against the ICDREC. For that purpose the ICDREC will have to be completed and its data verified. The clinical symptomatology of some case histories continues to await completion.

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Dose Estimation Using Lymphocyte Depletion Kinetics

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Introduction

Victims of high-level, whole-body gamma exposure have been shown to have an improved likelihood of survival if prompt and aggressive cytokine treatment is administered. However, in such cases both the magnitude of the accident and the dosimetry profile(s) of the victim(s) are often not known in detail for days to weeks. A simple dose-prediction algorithm based on lymphocyte kinetics is presented here. This algorithm provides an estimate of dose within the first 8 hours following an acute whole-body gamma exposure. This estimate may be used to guide initial medical treatment and may be modified as needed after more exact dosimetry becomes available.

Classically, physicians and health physicists have used the nomogram originally developed by Andrews [1,2] to predict the severity of the hematological component of the acute radiation syndrome. Figure 5 illustrates Andrews' proposed lymphocyte depletion kinetics, grouped by severity of exposure. This technique utilizes the absolute lymphocyte count at 24–48 hours post-accident to indicate broad ranges of dose, to correlate these ranges with a predicted clinical course, and to provide the treating physician with a guide to the magnitude of the

problem. From the REAC/TS Accident Registry, 43 examples of acute exposure have been selected where both lymphocyte kinetics and physical dose were considered to be well documented and reasonably reliable. These examples reflect not only our experience, but also that of colleagues in the former Soviet Union. In some cases the Soviet dosimetric data was obtained directly from accident reports in our Registry, and in other instances from personal communication with colleagues [3]. In addition, we have relied heavily on data gathered through interpolation of standard dose curves compiled from previously published summaries of the Chernobyl medical experience [4]. Medical details and physical-dose reconstruction of several of these accidents are found in two recent volumes on the medical management of radiation accidents [5,6].

Analytical Techniques

An empirical analysis of lymphocyte-depletion curves in many accident cases shows that lymphocytes disappear from the peripheral circulation after an acute
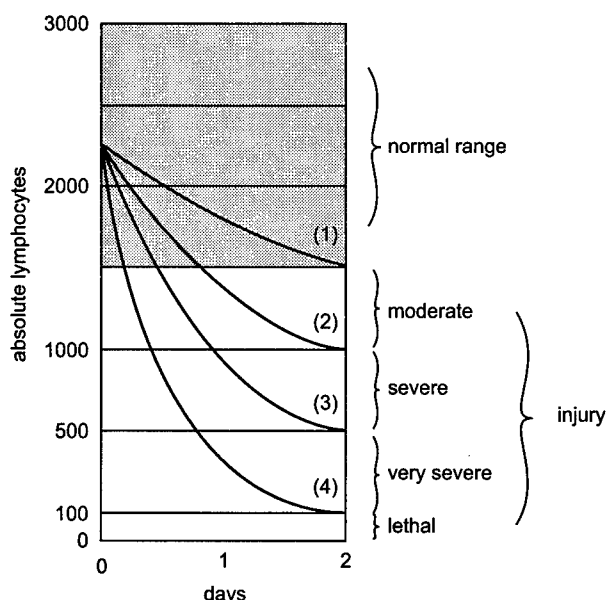

Fig. 5. Classical Andrews lymphocyte depletion curves and accompanying clinical severity ranges. According to the data presented in this paper, curves 1–4 correspond roughly to the following whole-body doses: curve 1 - 3.1 Gy; curve 2 - 4.4 Gy; curve 3 - 5.6 Gy; curve 4 - 7.1 Gy.

gamma exposure according to an equation of the form

$$L(t) = L_0 e^{-K(D)\,t} \qquad (1)$$

where L_0 is the lymphocyte count prior to accident, $L(t)$ is the count at time t post-accident, and $K(D)$ is a rate constant, dependent primarily on the average prompt dose, D. Equation 1 is a valid approximation for $t < 8$ hours post-accident and, in some cases, probably somewhat longer.

Figure 6a presents the complete range of data for the 43 cases that we have analyzed. The abscissa in figure 6a is the measured $K(D)$ for each accident; and the ordinate is the best estimate in the literature of whole-body dose, D, for that case. Relatively few cases with both good hematology and good dose estimates were available to us in the high-dose range. Therefore, the fit should be interpreted cautiously in this region. It should be emphasized that our simple analysis of lymphocyte kinetics is useful primarily as an early, rapid predictor of dose and not as a substitute for dose determined by more established techniques. Detailed analytical models of lymphocytopoiesis under both acute and chronic radiation regimens have been presented elsewhere [7,8 and references therein]. These models are valid for longer time intervals, contain various physiologically-based parameters, are more complete and, hence, more complex.

Using a commercially available, weighted Levenberg-Marquardt nonlinear regression routine [9] and weights equal to $1/\sigma_K^2$ for each K, it is possible to fit the data in figure 6a with a simple two-parameter dose response curve of the form:

$$D = \frac{a}{1+(\dfrac{b}{K})} \qquad (2)$$

where the parameters (mean $\pm$ SD) are found to be: $a = 13.6 \pm 1.7$ Gy, $b = 1.0 \pm 0.20$ days^{-1}, and $r^2 = 0.83$. The 99% prediction intervals for dose are also superimposed in figure 6a. An interesting observation of the two parameter fit is that a plot of $1/D$ versus $1/K$ is linear, and a weighted linear regression of $1/D$ versus $1/K$ gives essentially the same values for a and b as determined by the Levenberg-Marquardt method. From equation 2, it is easy to show that relative uncertainty in K propagates uncertainty in the estimate of dose as $(\delta D/D) = b/a(\delta K/K)$. Table 6 presents early dose estimates ($t < 8$ hours) along with the 99% prediction limits for a range of $K(D)$ using the two-parameter lymphocyte kinetic algorithm.

Approximately 75% of the cases under consideration involve whole body doses < 5 Gy, and this region is expanded in figure 6b. The lower dose region is also the one most likely to be encountered in practice, where k is relatively small and therefore $b/k \gg 1$. In this case, equation 2 reduces to $D \approx (a/b)K$ so that D and K are linearly related. With this fact in mind, it is logical to attempt to fit the lower dose data by a simple linear regression $D = eK$ with zero intercept. This has been done in figure 6b, with superposition of the 99% prediction intervals. The slope e of the weighted regression line in figure 6b is found to be 10.2 ± 0.40 Gy/day^{-1} with $r^2 = 0.75$.

Fig. 6. $K(D)$ versus D over the range 0–15 Gy. Fit is with the weighted two parameter transition model. The 99% prediction intervals are superimposed. Figure 6b is the linear portion of figure 6a. The weighted linear regression fit with 99% prediction intervals is superimposed.

Case History

A 34-year old male was accidentally irradiated in 1991 in Belarus in a ^{60}Co sterilization facility (~30PBq). Exposure to the source from variable distances lasted 1 to 2 minutes. Details of the medical and health physics of this accident have been reviewed elsewhere [10]. Nausea and vomiting occurred within 3 minutes after exiting the facility and diarrhea within 13 minutes. The patient also experienced the rapid appearance of the prodromal symptoms of tachycardia, hypotension, fatigue, fever, and headache. The initial medical assessment suggested a high-dose exposure and the victim was rapidly transferred to specialized facilities in Moscow. Our analysis of lymphocyte depletion curves within the first 8 hours, with a relatively good fit to a single exponential decay curve of slope $K = 2.48\ d^{-1}$, gives an initial dose estimate of 9.7 Gy with 99% prediction limits of 6.3 Gy and 13.1 Gy.

Detailed post-accident dosimetry was performed by various techniques [11]. Paramagnetic resonance analysis of the victim's dental enamel was consistent with a dose of 14.0 ± 0.7 Gy; and computer accident simulation indicated an approximate dose of 12.5 Gy (95% confidence limits: 10–15 Gy). Dose estimation using analysis of neutrophil kinetics was 9–11 Gy, while direct cytogenetic analysis of lymphocytes suggested a dose of 9.6 to 11.7 Gy. The pooled biologic data was reported as 9.9 Gy. Our early dose estimate is therefore seen to be in good agreement with results obtained by more established methods many days after the exposure.

Acknowledgment

Table 6. Estimated dose as a function of K.

K(D), day^{-1}	Estimated dose, Gy	Lower 99% limit	Upper 99% limit
0.0	0.0	0.0	0.0
0.05	0.65	0.50	0.80
0.10	1.24	0.98	1.50
0.15	1.78	1.44	2.12
0.20	2.27	1.87	2.68
0.25	2.78	2.27	3.18
0.30	3.15	2.66	3.63
0.35	3.53	3.02	4.05
0.40	3.90	3.36	4.43
0.50	4.54	3.97	5.12
0.60	5.11	4.50	5.73
0.70	5.61	4.95	6.28
0.80	6.06	5.33	6.79
0.90	6.46	5.66	7.25
1.0	6.82	5.95	7.69
1.5	8.18	6.90	9.46
2.0	9.09	7.43	10.74
2.5	9.73	7.76	11.71
3.0	10.22	7.98	12.45

(ORISE) was established by the U.S. Department of Energy to undertake national and international programs in education, training, health, and the environment. ORISE and its programs are operated by Oak Ridge Associated Universities (ORAU) through a management and operating contract with the U.S. Department of Energy. Established in 1946, ORAU is a consortium of 88 colleges and universities.

The Radiation Emergency Assistance Center/ Training Site (REAC/TS) was established in 1976 and is operated by the Environmental and Health Sciences Division of the Oak Ridge Institute for Science and Education (ORISE) in Oak Ridge, Tennessee, for the United States Department of Energy. The REAC/TS program provides 24-hour direct or consultative assistance regarding medical and health physics problems associated with radiation accidents in local, national, and international incidents.

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Fatigability and Weakness as Clinical Indicators of Exposure

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Introduction

The fatigability and weakness (FW) that humans frequently experience following exposure to ionizing radiation has led to a number of concerns. The two most apparent issues are 1) the degrading effects on performance capability in a military operational setting where personnel could be subjected to a nuclear-weapon environment, and 2) the debilitating side effects that accompany radiation

therapy in cancer patients. As such, the FW syndrome and its manifestation in humans has been the subject of study in an attempt to gain both a better qualitative and quantitative understanding of the phenomena.

In this presentation a review is given on 1) the clinical experience of radiation-induced FW (RIFW) that derives from data accumulated from radiation accident victims and from cancer patients who received radiation therapy; 2) the quantification of empirical response observations; and 3) possible causal mechanisms. Considering possible causal mechanisms of RIFW, a mechanistic modeling approach is selected to quantitatively gauge the response based on necessary, although not sufficient, conditions. Also, some comments are provided regarding the feasibility of utilizing RIFW observations in a combat operational setting as a "biodosimetry" approach to estimate the magnitude of radiation exposure in terms of body dose or exposure as an aid to define medical-care requirements. All dose magnitudes given in this presentation refer to midline tissue (MLT) of the body. Free-in-air (FIA) dose values at the outside surface of the body can be estimated by multiplying the MLT dose values by a factor of 1.5.

It is important to distinguish between fatigue and fatigability, relevant to task performance, in a military-operations setting. Both are symptomatic of ionizing radiation as has been observed in irradiated individuals; except that, the latter is revealed upon placing a demand on the body such as physical activity. That is, fatigue is a passive state that may or may not be experienced depending upon dose, physical condition, etc.; whereas fatigability requires some form of activity to experience reduced capability.

Clinical Experience

Based on medical records, the literature, and discussions with those who provide medical care for radiation-accident victims and radiation-therapy patients, a summary of the sources investigated are as follows:

- nuclear industry, radiation accident victims

- clinical radiation radiotherapy patients

- Japanese fishermen (Pacific Test fallout)

- 1984 Jarez accident

- Chernobyl accident victims

- Goiâna (Brazil) accident victims

- Oak Ridge Associated Universities/NASA studies.

The Intermediate Dose Program (IDP) sponsored by the Defense Nuclear Agency (DNA)** was responsible for gathering and analyzing the symptomatologic effects of RIFW caused by prompt radiation exposure (prompt radiation is radiation that is delivered in a very short period, at a rate of several hundred Gy/h or more). The findings and analysis of the DNA/IDP effort are given by Baum et al. [1] and Anno et al. [2]. Radiation-induced FW effects for protracted radiation were subsequently investigated under DNA's Human Response Program (HRP). The findings and analysis were given by Anno et al. [3] and Anno, McClellan, and Dore [4].

Various investigators over the years [e.g., 5–14] have all reported on the typical experience of RIFW for doses that range from approximately 1 Gy to over 6 Gy for both high- and low-dose rates from 0.6 cGy/h to well over 6 Gy/h as well as fractioned exposures. In the Court-Brown experience, halfbody irradiation of 3–4 megagram-r had a mean time to symptoms of 2.7 hours with reports ranging from sudden bouts of nausea or severe fatigue to mild or transitory nausea; fatigue without nausea was rare. Gerstner reported on the "typical" acute initial period radiation syndrome with upset stomach, anorexia, nausea, malaise, listlessness, drowsiness, and fatigue within 2 hours postirradiation, followed by a rapid deterioration of conditions leading to profuse vomiting, extreme weakness or even prostration, culminating in 8 hours and lasting 2 to 3 days. Miller et al. reported

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**Known as the Defense Special Weapons Agency (DSWA) since June 1996.

that after 132 rad (2.5 rad/min), 27 of 30 patients (90%) reported fatigue, decreased energy, drowsiness, or malaise within a few hours postirradiation, peaking at 6 to 8 hours, and subsiding 24 hours later. Bond stated that all individuals from accident cases (150 to 450 rads), reported a definite sense of fatigue which was coincidental with nausea and vomiting. Fatigue persisted in all cases for months. Rubin and Casarett reported that "muscle fatigue" was a common complaint in radiation therapy patients. The severest degree of creatinuria was observed in patients with severe fatigue. No direct effect of ionizing radiation on muscle was clinically recognizable. Lushbaugh reported the median lethal dose as 300 rad ± 100 rad, with the principal prodromal symtoms listed as anorexia, nausea, vomiting, and easy fatigability. Using physiologic monitoring and bicycle ergonometry, Lushbaugh demonstrated exercise intolerance and decreased performance capability for single prompt exposures (130 rad), for fractionated exposure (15 d x 6.7 rad = 100 rad) at the low-dose rate (1 rad/hr), and for continuous exposure (5 d x 20 rad/d = 100 rad) at the low-dose rate of 0.6 rad/hr. Hall reported principal prodromal symptoms as anorexia, nausea, vomiting, and easy fatigability at $LD_{50/60}$. Messerschmidt reported that in doses greater than or equal to 600 rad,

there was repeated fatiguing vomiting, nausea, and dizziness that were followed in a matter of minutes by drowsiness, and severe exhaustion. Circulatory symptoms leading to collapse were evident within a few hours. At 200 to 600 rad, nausea, vomiting, and exhaustion were reported as milder. Vodopick and Andrews, using data from a 127 rad Co^{60} accident, reported that the least amount of exertion led to excessive fatigue that continued for months. Endurance was tested using a bicycle ergonometer that charted the serum CPK level.

Thoma and Wald [15] gathered information on radiation accidents and provided a list of individuals who experience FW for doses that ranged from 22.8 cGy to 4500 cGy, their onset times, and duration (see table 7).

Kumatori et al. [16] gave the incidence of fatigue (~93%) in Japanese fishermen exposed to fallout radiation from a nuclear-weapon device exploded in the Pacific, where doses during the first day were estimated to range from about 130 to 450 cGy (see fig. 7).

Objective measurements of work capacity of Chernobyl accident victims [17] give the incidence

Table 7. Fatigability and weakness in accident victims.

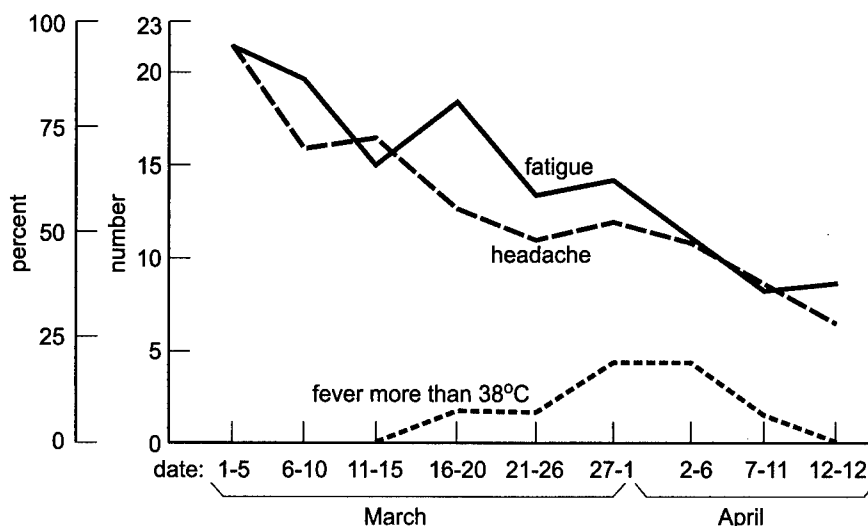| Case	Dose (rad)	Prodromal period		Manifest period	
		Onset	Duration	Onset	Duration
OR8	22.8	2 h	1 d		
Y6	145	1 d	> 120 d		
LA4	192	1 d	70 d		
Y5	226	1 d	> 120 d		
Y4	290	1 d	> 120 d		
Y2	293	1 d	> 120 d		
Y3	298	1 d	> 120 d		
R2	300	1 h	4 d	24 d	20 d
Y1	305	1 d	31 d		
LA1	310	1 h	24 d		
R1	450	1 h	4 d	19 d	22 d
LA3	1114	1 h	1 d	1 d	9 d
LA11	4500	5 m	35 h		

Fig. 7. Fatigue, headache, and fever - 23 Japanese fishermen.

and severity of asthenia 4 to 6 months after radiation exposure. Depending on the magnitude of the dose, 33% to 75% of the individuals exposed had asthenia which corresponded to a work capacity ranging from 61% to 88% of that normally expected (see fig. 8). Since these measurements were made months following the accident, reduced work capacity might be expected to have been even more prevalent at earlier times following the accident.

Quantification of Empirical Observations

Based on the clinical experience and analysis of the data and information gathered, the RIFW response in humans can be quantified in terms of a dose-response function and a time-dependent severity profile that can typically be expected to be exhibited by an individual exposed to various dose ranges of promptly delivered radiation.

group	dose (Gy)	asthenia incidence* tested 4-6 mos. post-accident	work capacity (Kcal/min)	
normal (no S/S)**	--		4.9 (342 watts)*** (100%)	
ARS 1	1-2	1/3	4.3 (300 watts) (88%)	ARS 1,2 levels returned to normal in 8-9 weeks and remain high at end of year
ARS 2,3	2-4	1/2	3.0 - 3.8 (201 watts) (61 - 78%)	
ARS 3	4-6	3/4	initial: 3.8 (265 watts) early recovery phase: 3.5 (244 watts) (71%)	

*Somatic basis for asthenia passed in 9 -12 months (recovery in the function of main organs); definite improvement in 3/4 in 2nd year post-accident.

**Signs/Symptoms

***Moderate activity: badminton, horse-back riding (trotting), square dancing, volleyball, roller skating.

Fig. 8. Work capacity - Chernobyl accident victims.

Table 8. Lognormal distribution parameters for FW.

$ED_{10} = 57.97 \ (+67.04, -31.04)^e$

$ED_{50} = 138.17 \ (+66.03, -44.63)^e$

$ED_{90} = 329.30 \ (+200.38, -124.58)^e$

$\mu = 4.9285^a$

$\sigma = 0.6776^b$

$\alpha = -7.273^c$

$\beta = 1.476^d$

$\chi^2 = 37.65$

$\nu = 44$ dgf

$p < 0.0005$

Dose summary[f]

Bin #	Number of cases	Response fraction	Mean dose (cGy)	Dose range (cGy)		
				Low	High	Probits
1	5	0.000	10.9	8.1	16.0	3.77
2	14	0.3571	92.1	22.8	192.0	4.38
3	6	0.6667	234.7	200.0	290.0	5.46
4	13	0.9231	327.5	293.0	450.0	6.16
5	8	1.000	1284.0	522.0	8800.0	13.37

[a]Mean natural logarithm of dose.
[b]Standard deviation of natural logarithm of dose.
[c]Probit intercept.
[d]Probit slope/natural logarithm of dose.
[e]95% confidence bounds.
[f]Used only to illustrate data, but not for probit likelihood calculations to fit the data.

Dose Response. The FW response data from 46 radiation accident victims exposed to promptly delivered radiation were used to perform a likelihood probit analysis to determine the dose-response function. The data and analyses are given by Anno, McClellan, and Dore [4] and are summarized in table 8.

A plot of the data is given based on the lognormal distribution response function along with the 95% confidence limits for the incidence response (see fig. 9). Grouped data that correspond to the dose bins given in figure 8 are also indicated in figure 9. Radiation-induced FW can be expected to initially occur at a relatively low dose (~58 cGy) although only in an estimated 10% of those exposed. The median dose for RIFW is estimated to be 138 cGy; 90-percentile dose is estimated to be 330 cGy.
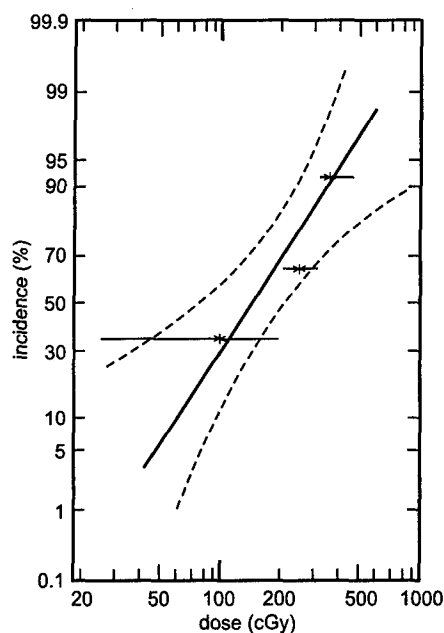
Fig. 9. Incidence of fatigability and weakness.

Table 9. Radiation-induced FW severity levels.

Severity Level	Description
1	No effect
2	Somewhat tired with mild weakness
3	Tired, with moderate weakness
4	Very tired and weak
5	Exhausted with almost no strength

Severity Profile. The severity of RIFW has been quantified in terms of descriptive levels of hierarchy based on five ordinal levels of increasing severity given in table 9. As part of the DNA/IDP studies, the basis for construction of time-dependent severity profiles are given (see fig. 10). These profiles parallel the empirical-response data. Information was extracted from the reviewed body of material and from the insight provided by radiation therapists, physicians who attended to accident victims, and clinical-care nurses. The clinical-care nurses were largely responsible for the care and well being of patients following exposure to radiation and were in some cases able to provide a detailed picture of RIFW.

As would be expected, the FW severity-response profiles show early onset of symptoms, increasing severity, and longer duration of symptoms with increasing dose. The response profiles are biphasic, which parallel the prodromal and manifest illness periods, typical of acute radiation sickness (ARS). RIFW is a ubiquitous response in humans who are exposed to ionizing radiation; onset, incidence, severity, and persistence depend to some extent on dose, and dose rate. The response is likely systemic in nature; it has been observed in total body irradiation (TBI), and in partial body irradiation to a somewhat lesser extent. RIFW can be a fairly sensitive indicator of radiation exposure since it can become apparent at a relatively low dose level, less than 100 cGy. The biphasic nature of the response which is initially present from hours to days and then again appears after an apparent period of remission, depending on dose level, can persist from weeks to months. This suggests at least two different (more than likely connected) causal mechanisms are responsible for the RIFW response in humans.

Possible Causal Mechanisms and Approach to Response Modeling

A variety of possible biological mechanisms that could cause RIFW include:

- Toxic substance accumulation (organ tissue damage/recovery)

*dose (FIA) = 1.5 dose (MLT)
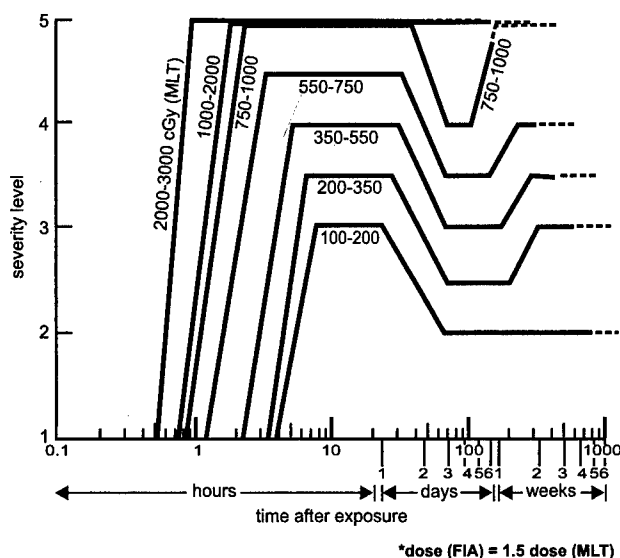
Fig. 10. FW empirical response profiler for dose ranges [MLT].

- Disrupted enzymatic processes involved in metabolism

- Direct and/or indirect effects of mitochondrial damage

- Impaired oxygen transport capability (RBC decline)

- Increased capillary/microvasculature permeability (endothelial damage)

- Damage to the neuromuscular junction

- Serotonin release stimulated by irradiation

- Endogenous cytokine release (immune system damage)

None of these have been isolated to account for the observed effect. However, the causal possibilities that have been suggested are based on observed temporal response and other biological indicators.

A review of fatigue in cancer treatment provided by nursing experience (see fig. 11) together with suggestions provided through a series of meetings held with radiobiologists, medical researchers, radiotherapists, physiologists, physicians, and oncology nurses have provided collective insight for an approach to modeling the RIFW response. Fatigability and weakness are not only present in radiotherapy (and chemotherapy) patients, but are also prevalent in biotherapy-treated cancer patients who were given interferon and interleukin. In fact, FW was so prevalent in many of these patients that it frequently became the dose- or treatment-limiting toxicity. Accordingly, we have theorized that the release of endogenous cytokines, modulated by radiation damage to the lymphatic system, represents a likely causal mechanism.

Our approach to modeling a radiation-induced response must satisfy at least two conditions: 1) the model must be based on a plausible biological system, organ, or tissue; and 2) the model must conform to empirical observations. It is well known that the lymphatic system is very sensitive to radiation; the lymphocyte count rapidly recedes following irradiation since the cells can die in interphase prior to cell division, normally characterized by other cells in

Clinical description
- A continuum ranging from tiredness to exhaustion (physical, mental, and emotional)

Incidence
- Radiotherapy (RT): 65% to 100%
  - chest RT: 93% (highest) 3rd week--46% 3 months postRT
  - pelvis (male) RT: 65% (lowest) last week--14% 3 months postRT

- Chemotherapy (CT)
  - breast (female) CT:96%
  - other CT: 59% to 82%

- Biotherapy (BT)
  - interferon: 74% dose, or treatment-limiting toxicity
  - interleukin: 71%

Persistence
- Can continue to be problem up to several months posttreatment

Measurement
- Mainly self-report measures (subjective experience); questionnaires, checklists (Likert-type) and visual scales, etc.

- Direct exercise activity measurements rare, some difficulty relating to level of fatigue

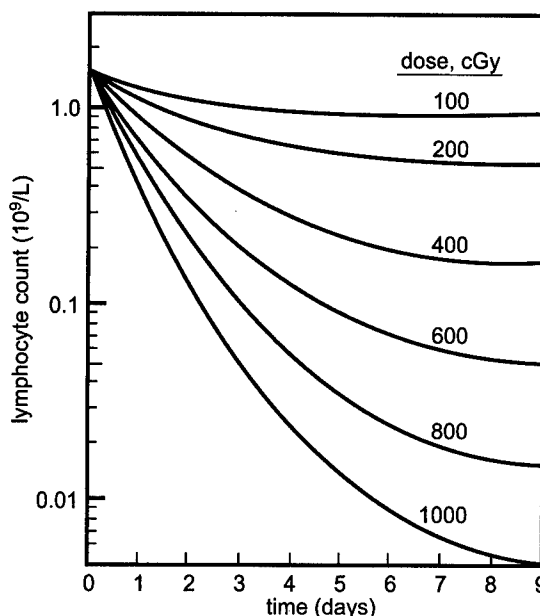**Fig. 11.** Fatigue in cancer treatment [18,19].

the body (see fig. 12). This temporal behavior is quite consistent with the observed onset of the initial phase of RIFW. The impetus for focusing on the lymphocyte/cytokine release approach as a RIFW response modeling paradigm is based on necessary conditions, which include:

- Release of endogenous cytokines modulated by lymphatic damage

    –IFN-α, IFN-β, and IFN-γ
    –IL-1, 2, 3, 4, 5, 10, and 13
    –TNF-α and TNF-β

- High radiation sensitivity of lymphocytes (D_o = 50 to 100 cGy)

- Interphase death of cells (apoptosis/necrosis)

- Correlates well with observed early fatigability/weakness response

- Late phase is related to inflammatory response originating from earlier cytokine-mediated action

- Approach emulates a complex process that currently cannot be sufficiently supported by specific research

The mathematical model for the RIFW response has been completed and resides in the RIPD code [20] as one of the computational algorithms that addresses the residual performance level capability for military task performance based on the FW sign/symptom category. The overall computational algorithm includes the basic lymphopoiesis model and equations formulated by Zukhbaya and Smirnova [21] coupled to the cytokine release model for the RIFW response based on the equations given by Anno, McClellan and Dore [4].

Fig. 12. Lymphocyte count after single acute gamma irradiation.*

*based on Guskova et al., 1988

Conclusions

Empirical experience has shown that RIFW will continue to present in individuals exposed to both prompt and protracted ionizing radiation. Reasonable semi-empirical models have been developed to predict both the incidence and time course of RIFW severity. These models will help to gauge the performance capability of military personnel who may be exposed to radiation in an operational setting.

The empirical response data for both incidence and severity are largely based on subjective indicators of RIFW which were described by irradiated individuals and in turn reported by first- and second-hand investigations. An attempt has been made to crudely quantify the empirical experience based on data gathered and interpreted. Quantification of the RIFW-incidence response, based on probit analysis of the data, depends on whether FW was reported as experienced by the irradiated individual (patient) or observed and reported by attending medical personnel. The same is true regarding the information describing RIFW severity, but with the added dimension of extent or level.

Clearly, attempting to quantify largely subjective information and data in the manner described above introduces considerable uncertainty in transforming word descriptions into numbers. With this in mind, estimates of RIFW for given dose levels can only be made in the "forward manner." Utilizing observations

of RIFW, based on of some level of degraded performance, to infer a corresponding dose level in the adjoint or "reverse manner" would involve even greater uncertainty than in the forward manner. For example, in order to make an estimate of dose level based on incidence, information would have to be gathered from a considerable number of exposed individuals *in situ*, as to whether vomiting occurred. This information would then have to be applied to the dose-response function to infer dose. It would be hard to imagine this as practical in a battlefield or conflict situation. Utilizing the RIFW severity profiles in a similar manner to infer dose level offers even a larger level of uncertainty because of the interpretation of the level of severity. Therefore, application of the RIFW response as a biodosimetry approach would not engender any reasonable degree of confidence. Radiation induced FW response in an operational sense would be somewhat feasible for a crude level of dose inference (e.g., low, medium, and high), or if used in this manner in conjunction with a scoring system that takes a variety of other response endpoints into account as a collective means to infer dose level.

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Appendix C

Session III

Large Animal Radiation Experiments

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Military radiation exposure scenarios during the cold war era emphasized exposures to nuclear weapons and the resulting fallout. Multiple exposures were a possibility as was the requirement for operations in areas contaminated by fallout. A model was needed to predict human responses including incapacitation, lethality, and prodromal symptoms as a function of dose, dose rate, and radiation quality, after uniform or non-uniform exposures. Pursuant to modeling, a wealth of information on acute lethality responses in several animal species was collected at several laboratories prior to 1975—at which time the U.S. large animal radiobiology programs were shut down.

This presentation focuses primarily on results from an interspecies comparison program conducted for more than a decade at the U.S. Naval Radiological Defense Laboratory (NRDL) in San Francisco. Testing a model originally proposed by Henry Blair from the University of Rochester, the split-dose methodology was utilized to evaluate "recovery" from radiation injury among several small (rodent) and large animal species. In this protocol, large numbers of animals were given a first dose that was 2/3 of the $LD_{50/30}$ or LD_{60}, and, at various times thereafter selected groups of animals were "challenged" with a second dose; the $LD_{50/30}$ or LD_{60} was then determined to measure the time-dependent disappearance of the injury produced by the first dose.

Results showed that interspecie differences were impressive. Mongrel dogs and Duroc swine recovered rapidly. Both showed that 50% of the initial damage had been repaired within 3 days, and that there was no delay in the onset. Swine showed an extensive increase in radiation sensitivity—with an $LD_{50/60}$ at 20 days that was 50% above normal. Sheep showed an initial multi-day delay in the initiation of recovery (this was also observed in Rhesus monkeys in experiments conducted at the School of Aerospace Medicine in San Antonio). In other studies in which $LD_{50/60}$ values for sheep and swine over dose rates of about 4 to 600 R per hour were determined, a very large dose-rate effect was observed in swine; while sheep showed much less of an effect. Sheep were also used extensively in studies on combinations of acute and chronic irradiation. It was shown that a first dose of 155R (given at 510R/hr) significantly interfered with recovery processes during chronic exposure at 4 R/hr. Thus, the expected sparing effect of low dose rate was largely obliterated following an initial exposure at a high dose rate. Recovery was also evaluated in sheep when the first dose was given at 2 or 4 R/hr. The major interspecie differences observed were: $LD_{50/60}$ at high dose rates, the initiation of recovery, the extent and duration of induced radioresistance, and the effects of dose rate on $LD_{50/60}$. Based on these results, selection of the most appropriate experimental surrogate to model early human radiation responses remains elusive.

Estimating Lethality Risks for Complex Exposure Patterns

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Assessing the lethality risk for different radiation exposure histories is an important consideration in the triage of irradiated personnel. Examples of exposure histories include, prompt exposure, protracted exposure followed immediately by prompt exposure, prompt exposure followed after an interval by prompt exposure, etc. Because the lethality risk will be related to the level of medical support received (from either echelon I or echelon II Army medical facilities), the level of protection provided by the medical support must be accounted for when evaluating lethality risk.

Ideally, the lethality-risk evaluation should take into account complicating factors such as wounds and skin burns. Based on the results of lethality-risk evaluations for different radiation exposure scenarios, Army medical facilities (echelons I and II) could be evaluated to determine whether they are able to provide their intended level of support (i.e., more staffing may be needed for some exposure scenarios). Lethality-risk evaluations for radiation-exposure scenarios could be used to obtain the information needed to revise the Army field manuals which are used in echelon I and II medical facilities and for contingency planning for medical staffing requirements.

The Weibull normalized-dose model (or hazard-function model) can be used to evaluate the lethality risk for persons exposed to complex dose-rate patterns of gamma rays. This model can be adapted to account for: 1) an elevation in the lethality risk from wounds; 2) an elevation in the lethality risk from skin burns; 3) a reduction in the lethality risk resulting from the medical support received at a given level (Army medical facility echelon I or II); and 4) exposure to mixed neutron/gamma fields.

The Weibull normalized-dose model is dose-rate dependent and was developed using data from both laboratory animals exposed to gamma or x-rays and humans exposed to gamma rays. In the model normalized dose, X, is used as the independent variable. Normalized dose is simply dose expressed as a dimension-less fraction of the median lethal dose (LD_{50}) when lethality is the endpoint of interest. For example, X=0.5 (one half of an LD_{50}) is the current central estimate of the threshold for death via the hematopoietic syndrome mode (regardless of the dose-rate pattern over time) when humans are exposed over the total body to gamma radiation. It follows that X=1 corresponds to the LD_{50} for any complex, total-body exposure pattern of interest, when a single mode of death applies.

The normalized dose could be used to facilitate making decisions related to triage of irradiated personnel, provided personal physical dosimeters were worn during periods of radiation exposure and were read at suitable intervals. This useful feature of X arises because the risk of death via the hematopoietic-syndrome mode is uniquely determined by X, irrespective of the complexity of the exposure pattern considered. The risk, R, of lethality from a given mode (hematopoietic-syndrome mode here) is related to X through the equation $R = 1 - \exp(-0.6931X^V)$, where V is a fixed parameter called the shape parameter. For the hematopoietic-syndrome mode of death, V has been estimated to be 6 (with lower and upper bounds of 4 and 8, respectively). For values of X less than a threshold, X_o, R is discounted (treated as being zero) for the lethality endpoint. For the hematopoietic syndrome mode of death, X_o has lower-bound, central, and upper-bound estimates of 0.4, 0.5, and 0.6, respectively.

For the hematopoietic-syndrome mode of death, analytical solutions have been developed for X for different gamma-ray, dose-rate patterns to the total body that include: 1) single, short-term exposure at a constant rate (including prompt exposure); 2) prompt (or protracted) exposure at a constant rate followed by protracted (or prompt) exposure at a constant rate; 3) exposure in a fallout field where the gamma-ray dose rate decreases as $At^{-1.2}$, where t is hours after arrival of the fallout and A is a positive parameter; 4) exposure to internally incorporated gamma-emitting radionuclides, where the dose rate decreases as a single, negative-exponential function

of time—as can occur with radiolabeled-antibody therapy for cancer; and, 5) prolonged exposure (over several years) at very low, steadily declining dose rates, similar to the exposure pattern for workers at the Mayak nuclear facility in the former Soviet Union. Mayak workers in some cases received protracted gamma-ray doses as high as 10 Gy which were insufficient for causing lethality from the hematopoietic-syndrome mode; whereas prompt doses to atomic-bomb victims in Nagasaki as low as 2 Gy were likely sufficient for lethality from the hematopoietic-syndrome mode in some cases.

Analytical solutions obtained for the normalized dose X for prompt-plus-protracted and for protracted-plus-prompt, gamma-ray exposure scenarios are provided in table 1. Two recovery cases were considered: No recovery between the two exposures, and, full recovery between the two exposures. Results presented in table 1 for full recovery apply to cases where the first dose is near or below the threshold for death via the hematopoietic-

syndrome mode. For protracted exposure of the total body at a constant rate to gamma rays, followed shortly thereafter by prompt exposure of the total body to gamma rays, X can be evaluated as $X = X_{protracted} + X_{prompt}$. Here, normalized-dose increments are evaluated separately for protracted and prompt exposures and added. The increments in normalized dose are evaluated as $X_{protracted} = D_{protracted}/LD_{50,protracted}$ and $X_{prompt} = D_{prompt}/LD_{50,prompt}$, where D is used for absorbed dose to bone marrow, and LD_{50} is dose-rate dependent. Similarly, when dose rate continually changes over time (as occurred for Mayak workers), increments in X can be evaluated for small increments in time (accounting for dose-rate changes over time, as above, using dose-rate-dependent LD_{50}). Increments in X obtained this way can then be added to give the total X.

Based on the analytical solution for X that applies to exposure at a constant dose rate r (in Gy/h to bone marrow), the number of hours of exposure required to reach the threshold X_o for death via the

Table 1. Analytical solutions for the normalized dose for the hematopoietic-syndrome mode of death, for complex gamma-ray exposure patterns.

Exposure pattern	Analytical solution for X	Comment
Constant dose rate	$X_{prompt} = D_{prompt}/\theta_\infty$	Prompt exposure
	$X_{protracted} = D_{protracted}/\theta(d_{protracted})$	Protracted exposure
Prompt + prompt	$X_{prompt} + X_{prompt}$	No inter-fraction recovery
Prompt + prompt	$(X^V_{prompt} + X^V_{prompt})^{1/V}$	Full inter-fraction recovery
Prompt + protracted	$X_{prompt} + X_{protracted}$	No inter-fraction recovery
Prompt + protracted	$(X^V_{prompt} + X^V_{protracted})^{1/V}$	Full inter-fraction recovery
Protracted + prompt	Same as for prompt + protracted	Full or no recovery

Where:

D is the absorbed dose (in Gy) to bone marrow; D_{prompt} and $D_{protracted}$ are prompt and protracted doses, respectively;

V is dimension-less and has lower, central, and upper estimates of (4, 6, 8);

d is the dose rate to bone marrow in Gy/h;

$d_{protracted}$ is the constant, protracted, dose rate to bone marrow in Gy/h;

$\theta(d)$ is the dose-rate-dependent LD_{50}, where $\theta(d) = (\theta_1/d) + \theta_\infty$;

θ_1 is in Gy^2/h and has lower, central, and upper estimates of (0.06, 0.072, 0.084)

θ_∞ is in Gy and has lower, central, and upper estimates of (2.5, 3, 3.5).

Threshold normalized dose has lower, central, and upper estimates of (0.4, 0.5, 0.6).

For full recovery cases, the first dose X must exceed the threshold to be counted; if the first dose is less than the threshold and the second dose is also less than the threshold, neither is counted.

hematopoietic-syndrome mode is given by $(X_o/r)LD_{50}$, where the LD_{50} in Gy is evaluated at dose rate r using the equation $LD_{50} = [(0.072/r) + 3]$. Also, the equivalent prompt dose (EPD) is obtained by multiplying X by $LD_{50,prompt}$. The $LD_{50,prompt}$ has lower-bound, central, and upper-bound estimates of 2.5, 3.0 and 3.5 Gy, respectively.

The solutions in table 1 were used to evaluate lethality risks for protracted exposure over 1 week to 0.7 Gy gamma rays followed immediately (or after an interval that allows full recovery) by prompt exposure to 0.5, 1.5, or 3 Gy doses. Similar evaluations were carried out for corresponding cases where the 0.7 Gy is delivered as a prompt dose. Using the solutions in table 1, lethality risks were evaluated for the indicated gamma-ray exposure scenarios. Uncertainties were evaluated using the Monte-Carlo method. Thus, rather than obtaining a single risk for a given scenario, risk distributions were generated to facilitate evaluating model uncertainty. Lower-bound, central, and upper-bound estimates of risk are presented in table 2 for the scenarios considered. The lower and upper bounds are based on the 5 percentile and 95 percentile of the risk distribution, respectively.

Similar calculations were carried out for a protracted dose of 1.5 Gy delivered over 1 week followed by a prompt dose of 0.5 Gy. Results indicate that for both the full- and no-recovery cases, the 1.5 Gy (protracted) plus 0.5 Gy (prompt) dose combination poses essentially no risk of lethality. However, nausea, vomiting and other prodromal symptoms are likely to be complicating factors. These results as well as those presented in table 2 apply to cases where the gamma-irradiated persons do not have wounds or skin burns and do not receive supportive medical treatment. The risks in table 2 would be expected to be reduced by supportive

Table 2. Lethality risks for gamma-ray, two-dose, fractionated exposure scenarios: First dose = 0.7 Gy, second dose = 0.5, 1.5, or 3.0 Gy.

Exposure scenarios					Central risk estimates and lower and upper bounds[a]		
Type of exposure[b]	Inter-fraction recovery	1st dose (Gy)	2nd dose (Gy)	Total dose (Gy)	Lower bound[c]	Central[c]	Upper bound[c]
Protracted + prompt	Full	0.7	0.5	1.2	0	0	0
	None	0.7	0.5	1.2	0	0	0
	Full	0.7	1.5	2.2	0	0	0.030
	None	0.7	1.5	2.2	0	0.016	0.042
	Full	0.7	3.0	3.7	0.30	0.50	0.76
	None	0.7	3.0	3.7	0.36	0.57	0.83
Prompt + prompt	Full	0.7	0.5	1.2	0	0	0
	None	0.7	0.5	1.2	0	0	0
	Full	0.7	1.5	2.2	0	0	0.030
	None	0.7	1.5	2.2	0.046	0.10	0.21
	Full	0.7	3.0	3.7	0.30	0.50	0.76
	None	0.7	3.0	3.7	0.71	0.91	1.00

[a]Generated by the Monte-Carlo method. Lower and upper bounds are based on 5 and 95 percentiles for risk distribution.
[b]Protracted first doses delivered over 1 week (7 days). All doses are to bone marrow.
[c]For full recovery scenarios, risk totally due to 2nd dose.

medical treatment received at Army medical facilities (echelons I and II). The risks would be expected to be higher for persons with wounds and/or skin burns.

The normalized dose, X, can be adjusted to account for: 1) protection provided by medical support received at Army medical facilities (echelons I and II) by dividing X by a protection factor (PF), where PF ≥ 1; 2) the presence of wounds and skin burns by multiplying X by a susceptibility factor (SF), where SF ≥ 1; and, 3) the presence of neutrons (i.e., mixed neutron/gamma fields) through use of an appropriate neutron RBE for death via the hematopoietic-syndrome mode when calculating X. For no protection from radiation injury, PF = 1. For normal susceptibility to irradiation, SF = 1. However, additional research would be required to develop reliable factors (SF and PF) for exposure scenarios and for medical support that would be received at Army medical facilities.

The Weibull normalized-dose model also can be used for evaluating lethality risk from irradiation of the large or small intestine and the lung and for evaluating the morbidity effects from irradiation. The United Kingdom's National Radiological Protection Board has adapted the model and has applied it a number of morbidity effects from exposure to gamma rays. The model is also used in the computer code MACCS developed at Sandia National Laboratory for the U.S. Nuclear Regulatory Commission and is used in the corresponding European code COSYMA, used by the Commission of European Communities for assessing expected health effects from nuclear accidents that involve complex patterns of combined exposure to alpha, beta, and gamma radiations.

Acknowledgments

An Integrated, Physiologically Based Model of Human Response to Multiple Exposures

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The Radiation-Induced Performance Decrement (RIPD) computer code calculates the severity of illness for persons who receive either prompt or protracted exposures to ionizing radiation for total free-in-air doses between 75 cGy and 4500 cGy. The RIPD calculations apply to whole-body exposures of gamma rays and/or neutrons for complex time histories that involve exposure periods for up to 1 week duration.

The RIPD code also calculates the residual performance capability over time for exposed soldiers in a military engagement. The performance functions are intended for consequence assessment and military planning in a nuclear radiation environment. The response to exposure expressed in terms of severity of illness and performance capability is calculated for as long as 1000 h (about 6 weeks) after start of exposure.

The RIPD code is designed to answer questions regarding the human response to multiple exposures of ionizing radiation. This short report summarizes the modeling approach used in the RIPD code, provides references to detailed descriptions of the model components, and provides sample RIPD calculations.

Modeling Approach

Figure 1 illustrates the modeling approach used in the RIPD code. The flexibility to accept any exposure sequence derives from the use of physiologically-
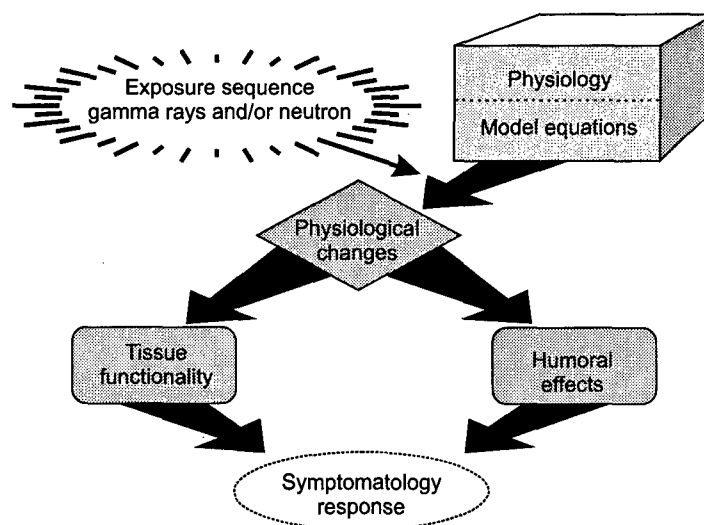
Fig. 1. The RIPD code uses physiologically-based models to estimate the human symptomatology caused by either prompt or protracted exposure to ionizing radiation.

based models that mathematically simulate the dominant responses of bodily systems to irradiation. These models employ the radiation dose rate R(t) in differential equations that mimic the kinetics of the bodily systems. The RIPD code numerically integrates these differential equations for any user-specified exposure sequence.

As illustrated in figure 1, the physiological models in the RIPD code are of two types, humoral and tissue-oriented. A humoral model is based on the kinetics of the production and metabolic clearing of toxins or neuroactive substances within bodily fluids. A tissue-oriented model is based on the structure and cellular kinetics of a primary tissue in an organ or organ system. For each model, a variable such as a toxin level or a cellular-population level determines the severity of symptoms.

The RIPD code uses a quantitative description of the sign/symptom severities of acute radiation sickness (ARS) developed for the Defense Special Weapons Agency (formerly the Defense Nuclear Agency) by the Intermediate Dose Program (IDP) in the early 1980s. The IDP methodology divides the major signs and symptoms of ARS into six categories [1,2]:

1. Upper Gastrointestinal Distress (UG),

2. Lower Gastrointestinal Distress (LG),

3. Fatigability and Weakness (FW),

4. Fluid Loss and Electrolyte Imbalance (FL),

5. Infection and Bleeding (IB), and

6. Hypotension (HY).

The RIPD code calculates a severity level for each of the six sign/symptom categories on a scale of 1 to 5. In addition, the RIPD code estimates the incidence of UG and FW symptoms, the incidence of mortality, and the typical time of mortality. In the context of signs and symptoms, incidence refers to the percent of a population in which the signs/symptoms are expressed; severity refers to the typical degree of illness of those having the signs/symptoms.

Model Structure

Figure 2 illustrates the model structure of the RIPD code. There are both stand-alone models, that require only the exposure dose rate as input, and slaved models that require input from the stand-alone models. The severity levels for the first three symptom categories listed above (UG, LG, and FW) are based on stand-alone kinetic models developed at Pacific-Sierra Research Corporation

[3–5]. Likewise, the incidence of mortality is based on a stand-alone cell kinetic model of myelopoiesis [6] developed at Oak Ridge National Laboratory (ORNL). The slaved models are represented in figure 2 by ovals with input arrows from the relevant stand-alone models. For example, the fluid-loss model for the prodromal phase of ARS requires input from both the lower and upper gastrointestinal distress models. The severity of FL symptoms is calculated using a reservoir model of bodily fluids that are disturbed by fluid loss from vomiting and diarrhea (as represented by UG and LG severity, respectively).

As indicated in figure 2, the models for UG and FL symptom severity each have two components. The two components correspond to the prodromal and manifest illness phases of ARS. The earlier prodromal phase is based on the stand-alone models discussed above. The later manifest illness phase is caused by the deterioration of the immune system that results from radiation damage to the bone marrow. The manifest illness phases of the UG and FL symptoms are slaved to the ORNL lethality model through an equivalent prompt dose (EPD). For a given protracted exposure sequence, the EPD is the single prompt gamma dose that produces the same cell population nadir in the ORNL myelopoiesis model as the protracted sequence. The incidence of lethality for the protracted exposure sequence is determined by the EPD and the (lognormal) dose-

response curve for prompt exposures. Similarly, the UG and FL severity levels during the manifest illness phase for a protracted exposure are determined by the IDP prompt dose severity profiles [2] using the EPD from the mortality model. Finally, the IB and HY symptom-severity levels are obtained from the IDP prompt-dose profiles using the same EPD.

The stand-alone model for the UG severity level is based on the production and clearing of humoral toxins [5]. The LG severity level is based on a cellular kinetics model of the intestinal mucosa [4]. The FW severity level is based on the killing of lymphocytes and the resulting cytokine production [5].

Each of the kinetic models used in the RIPD code is based on sound radiobiological principles, some of which are derived from animal studies. In each case, however, parameters of the models have either been taken directly from human tissue studies or adjusted so that the output of the models matches the human responses derived from accidental exposures and radiation therapy. The pitfalls of extrapolating the details of specific biological endpoints for radiation exposure from one species to another, particularly from animals to man, are numerous and well known. Generally speaking, however, all of
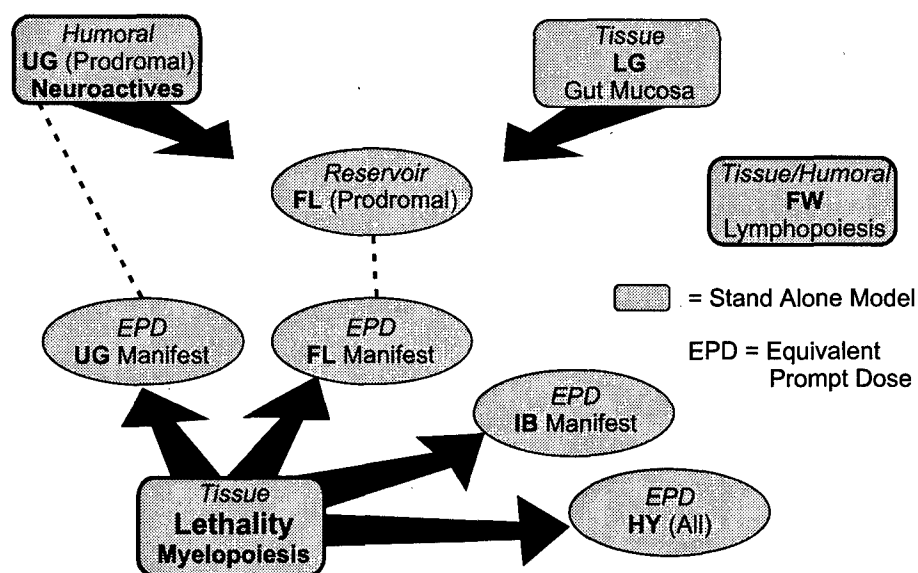

Fig. 2. The model structure of the RIPD code is built around four stand-alone kinetic models.

the principles of radiation biology have been eluci-dated by observations of the radiation response of nonhuman species. Furthermore, the radiotherapy community is quite successful in translating the re-sults of animal and cell-line studies into effective protocols for treating, with fractionated and con-tinuous exposures, cancer in humans. The RIPD models are built on that success.

Performance

In addition to a quantitative description of ARS, the IDP effort generated functional relationships be-tween the severity of ARS and the degradation of soldier performance in relation to various military tasks [7]. These performance functions are built into the RIPD code [8]. Briefly, the performance for a selected task is a logistic function of a linear combination of the severities of the six basic sign/symptom complexes. Performance is defined as the baseline time for a healthy soldier to complete a task divided by the time taken to complete the task when ill. It ranges in value from 1.0 for a healthy soldier to 0.0, which indicates that the soldier is too ill to do anything. The U.S. Army Nuclear and Chemical Agency refers to soldiers with perform-ance scores between 1.0 and 0.75 as combat effec-tive; those with performance scores between 0.75 and 0.25 as performance degraded; and those with scores below 0.25 are considered combat ineffective.

Prior-Dose Effects

To quantify prior-dose effects, it is generally necessary to 1) select representative multiple-dose scenarios, and 2) select the medical or per-formance endpoint of interest. Table 3 lists re-sults of RIPD calculations showing the influence of the recovery interval between a pair of 150 cGy doses (all doses quoted herein are free-in-air). The incidence data shows that a few days re-covery after the first dose greatly reduces mor-tality and significantly reduces the incidence of UG symptoms but has little impact on the inci-dence of FW. In each case the nadir of perform-ance occurs after the second dose. A day or two of recovery mitigates performance degradation severity.

Table 3 illustrates the changing response with fixed dose for the varying recovery interval. Table 4 shows the results of RIPD calculations where the dose is varied to cause a fixed response for each recovery interval; for example, 10% mortality requires a dose of 410 cGy for a 1-week recovery interval between an equal pair of doses. With no recovery interval between doses, only 274 cGy is required.

Table 3. Effect of recovery interval on various endpoints for a pair of 150 cGy doses.

Recovery interval	Incidence (%)			Performance nadir (physically demanding)		
Days	Mortality	UG	FW	Performance	Time (h)	Perf. at 1000 h
0	15.0	73	71	0.56	8	0.77
1	7.4	44	69	0.65	32	0.77
2	5.9	35	64	0.67	56	0.77
4	4.0	35	71	0.66	104	0.75
7	2.4	40	71	0.63	177	0.73
150 cGy acute dose	0.7	13	32	0.71	9	0.80

Table 4. Isoeffect total doses for an equal pair of dose fractions with varying recovery intervals.

Recovery interval	Isoeffect dose (total, in cGy) for four endpoints			
Days	Latent ineffectiveness[a]	LD_{10}	50% incidence of degradation[b]	Performance nadir of 0.75
0	402	274	206	125
1	451	321	212	168
2	490	337	228	184
4	610	368	206	183
7	620	410	207	167

[a]Latent ineffectiveness means: (a) that performance drops below 75% within 3 hours of the start of exposure and remains below 75% for 1000 h, or (b) that performance drops below 25% anytime within 1000 h after start of exposure.

[b]Incidence of degradation is defined as the greater of the incidence of UG symptoms for the given exposure. Performance degradation is conditional on the occurrence of the symptoms.

Tables 3 and 4 give a brief introduction to the calculations that may be done with the RIPD code. Many others are possible. For example, Radiation Exposure Status (RES) categories are based on Army doctrine—in part on the incidence of prodromal symptoms at low doses and on the desire to limit casualties after multiple exposures. The status of a unit is used by the commander to manage risk for the next mission. Because the RIPD model estimates incidence of prodromal symptoms for protracted exposures, it can be used to estimate RES categories for multiple exposures.

Conclusion

In summary, radiation dose rate and the occurrence of multiple radiation exposures are important considerations in operational planning. The Radiation Induced Performance Decrement software is available to support consequence assessment and military planning in a nuclear-radiation environment for both performance-based assessments and medical-casualty assessments for acute radiation sickness.

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Operational Performance Decrement After Radiation Exposure

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Introduction

The process of estimating the decrement in military task performance after exposure to ionizing radiation in an operational setting is presented using data from the Intermediate Dose Program (IDP) investigations dating from the early 1980s, sponsored by the Defense Nuclear Agency (DNA)[*]. The methodology developed to quantitatively predict a degraded level of performance is based on the residual performance capability of a combat soldier, estimated with a performance algorithm that depends on the severity levels of the known signs/symptoms (S/S) of acute radiation sickness (ARS). Because of the variability in human response, the uncertainty in performance level

that results from the physiological stress from exposure to radiation is also addressed.

Nausea and vomiting are present as the earliest and most obvious prodromal response indicators of ARS. As such, they can be expected to be largely, although not wholly, responsible for degraded performance, particularly during the prodromal phase. Accordingly, considerable effort was applied by the Defense Special Weapons Agency (DSWA) and the NATO Army Armaments Group (NAAG), Project Group 29 (PG-29) to promote promising antiemetic drugs (Ondansetron and Granisetron) to ameliorate early nausea and vomiting. To illustrate the efficacy of such drugs in terms of performance level, some bounding calculations were performed with the RIPD code [1]. These calculations presume either zero or, ideally, 100% effectiveness in ameliorating the upper gastrointestinal (UG) distress[**].

DNA Methodology

The DNA/IDP methodology evolved as a multistep process that involved a series of investigations, the results of which were integrated into a system of algorithms and databases. These were then used to predict degraded performance caused by bodily injury and stress from radiation or chemical agent exposure (fig. 3). The foundation for the methodology was established in a comprehensive effort to analyze the symptomatology of ARS and was used to categorize and quantify the S/S in terms of onset, duration, and severity [2–4]. This effort produced S/S complexes that are the elements of a dose × time ARS S/S map. Questionnaires were designed for selected combat personnel who were subject matter experts (SMEs) in their specialties. The SMEs provided response data about their ability to perform military tasks if subjected to radiation stress in a combat situation. The questionnaire responses and data analysis provided the quantitative link (and statistical parameters) from ARS S/Ss to degraded

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*Known as the Defense Special Weapons Agency (DSWA) since June 1996.
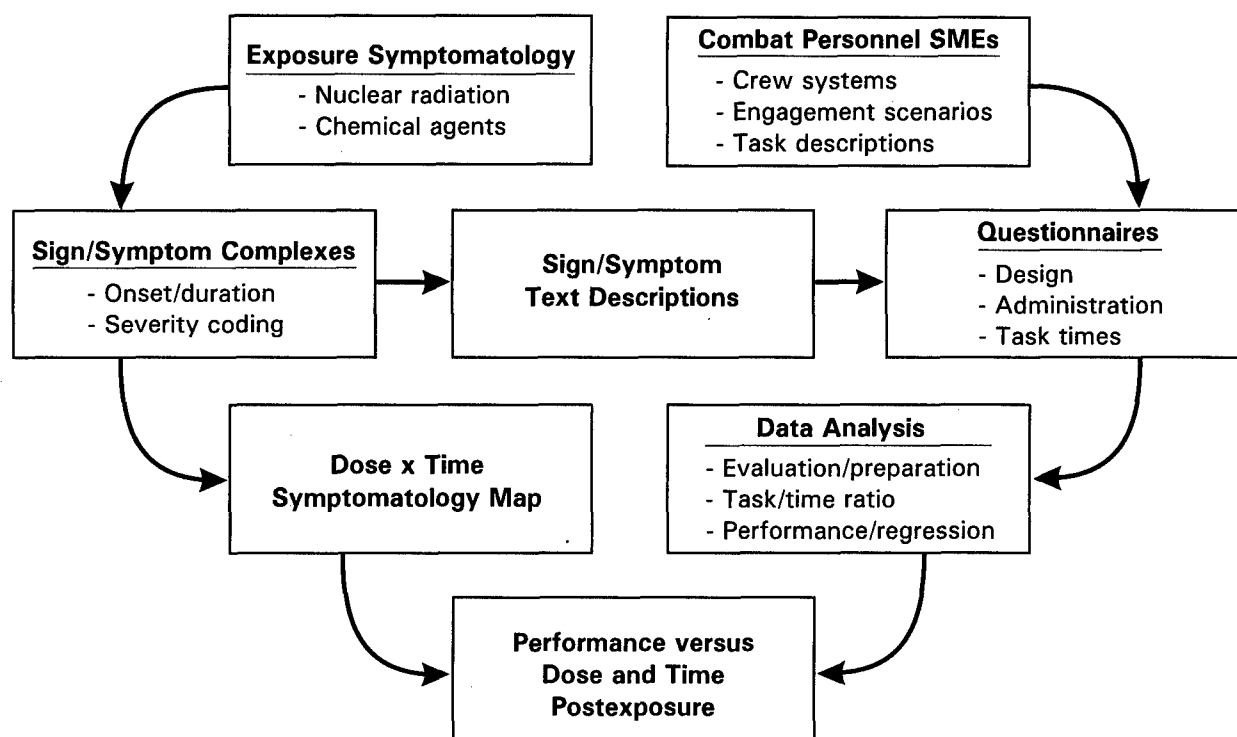**UG distress refers to nausea and vomiting.

Fig. 3. DNA/IDP methodology.

performance level. The SMEs were military personnel from the enlisted ranks who represented various combat crews or units (fig. 4). Each type of questionnaire contained 30 to 40 separate verbal descriptions of different S/S complexes. The S/S complexes define the dose- and time-dependent state of ARS given by the symptomatology map

(fig. 5). Each questionnaire also contained descriptions of different combat tasks common to each type of combat crew or unit, which were all familiar to the respective SME personnel. The SMEs offered their judgments as to the added difficulty in task performance introduced by ARS. The sympton complex descriptions were expressed in

Combat Crew/Unit	Data Origin	No. Respondees/Agent*			No. Tasks/Agent*		
		NR	GB	HD	NR	GB	HD
M-109 (155 mm) SP Howitzer	Ft. Sill	25	-	-	11	-	-
Fire direction center	Ft. Sill	21	-	-	8	-	-
M60A3 tank	Ft. Knox	34	-	-	10	-	-
M-901 ITV TOW	Ft. Benning	36	-	-	7	-	-
Dismounted infantry	Ft. H-Lig.	29	27	27	8	8	8
M 119A1 (105 mm) Lt. Howitzer	Ft. H-Lig.	-	20	20	-	9	9
Totals		**145**	**47**	**47**	**44**	**17**	**17**

*NR = Nuclear Radiation
GB = Sarin
HD = Mustard

Fig. 4. Combat task performance data from questionnaires.

Fig. 5. Sign/symptom complexes for exposure to nuclear radiation—symptomatology map.
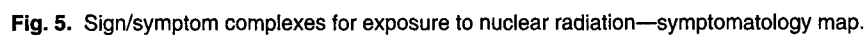
UG	LG	FW	HY	IB	FL
1	1	3	1	2	1

Tired, with moderate weakness; mild fever and headache; flu-like symptoms

UG	LG	FW	HY	IB	FL
5	1	4	1	1	1

Repeat vomiting with dry heaves; severe nausea and vomiting; fatigue and weakness

Fig. 6. Sign/symptom complex examples—nuclear radiation sickness.

plain language coded with numbers that ranged from 1 to 5 (fig. 6). The coded digits express the level of severity for each of the six S/S categories (UG for upper gastrointestinal distress, LG for lower gastrointestinal distress; FW for fatigability and weakness; HY for hypotension; IB for infection and bleeding; and FL for fluid loss/electrolyte imbalance). Each severity level is associated with a different descriptive phrase to denote differences in S/S severity. For a given S/S category, the severity-level descriptions designate a progression in S/S severity such as for nausea and vomiting (see table 5).

Dose Response. The dose response for the incidence of nausea and vomiting in the first 24 hours after exposure is estimated from quantal-response data from 46 different accident cases that involved prompt or high dose-rate radiation exposure, i.e., in excess of several hundred centigray per hour. The accident-case data included doses that range from 8.1 to 8800 cGy. Employing the lognormal distribution model, a likelihood/probit analysis with the quantal data was performed. Table 6 provides the dose-response function parameters obtained from the probit analysis. The analysis indicates that both nausea and vomiting are well correlated as to

Table 5. Severity levels of upper gastrointestinal distress in humans.

Severity level	Signs and symptoms
5	Vomiting and retching several times including the dry heaves; severely nauseated and will soon vomit again.
4	Vomiting and/or retching once or twice; nauseated and vomiting may recur.
3	Nauseated with considerable sweating and frequent retching and swallowing to avoid vomiting; a small percent will vomit.
2	Upset stomach; clammy and sweaty; may experience frequent swallowing; mild nausea; vomiting likely only in the most sensitive.
1	Normal; no noticeable effect.

Table 6. Lognormal distribution parameters for nausea and vomiting.

Parameter	Nausea	Vomiting
μ	4.9802[a]	5.1408[a]
σ	0.4345[b]	0.3925[b]
α	-11.462[c]	-13.098[c]
β	2.302	2.548[d]
$\chi 2$	45.82	34.95
ν	44 dgf	44 dgf
ρ	< 0.0005	< 0.0005
ED_{10}	83.37 (+62.47, -35.71)[e]	103.31 (+66.72, - 40.54)[e]
ED_{50}	145.50 (+53.19, -38.95)[e]	170.85 (+52.61,-40.22)[e]
ED_{90}	253.94 (+96.82, -70.10)[e]	282.56 (+98.87, -73.24)[e]

Data Summary[f]

Bin #	Number cases	Response fraction		Mean dose (cGy)	Dose range (cGy)		Probits	
		Nausea	Vomiting		Low	High	Nausea	Vomiting
1	7	0.000	0.000	10.9	8.1	42.0	3.01	2.74
2	10	0.300	0.100	92.1	60.5	159.2	4.18	16.25
3	6	0.833	0.833	234.7	165.0	236.0	5.58	5.23
4	13	0.923	0.923	327.5	270.0	365.0	7.01	6.62
5	10	1.000	1.000	1284.0	410.0	8800.0	16.96	16.25

[a]Mean natural logarithm of dose.
[b]Standard deviation of natural logarithm of dose.
[c]Probit intercept.
[d]Probit slope/natural logarithm of dose.
[e]95% confidence bounds.
[f]Used only to illustrated data, but not for probit likelihood calculations to fit the data.
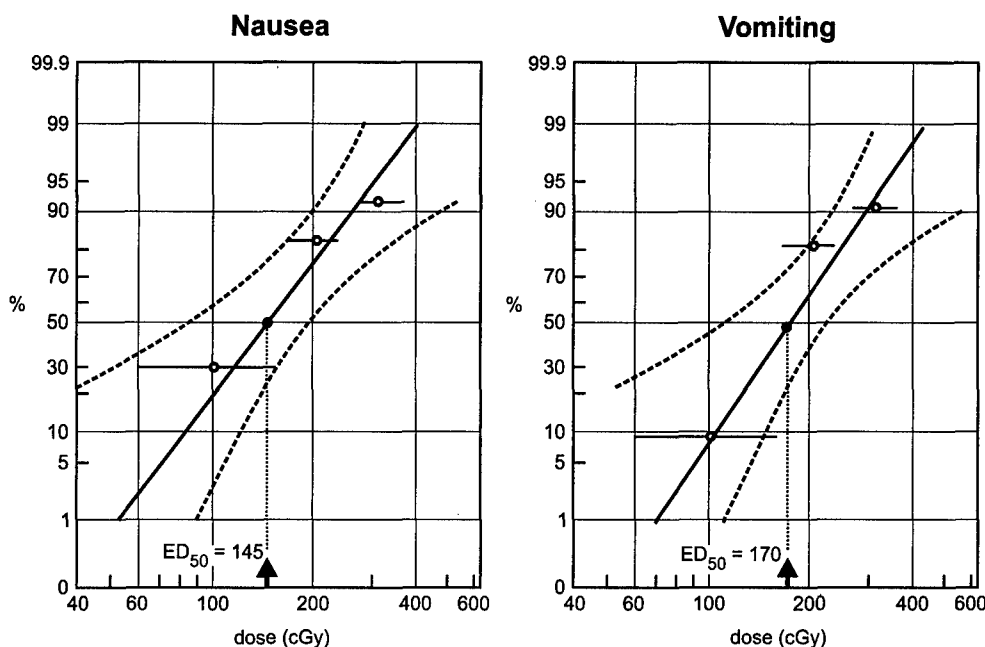
Fig. 7. Percent of incidence for upper gastrointestinal distress.

incidence (fig. 7). That is, rarely does vomiting occur that is not associated with nausea according to the accident data. The dashed lines on the plots of the dose-response function are the 95% confidence limits.

Onset of Vomiting. The onset of vomiting is dose dependent (see fig. 8). Regression analysis of the onset of vomiting was performed with data from radiation accidents, radiation therapy patients, expert opinion, and composite sources. The latter are estimates made in various summary documents and

guidance manuals that reflect a high degree of uncertainty, indicated by the solid lines connecting the open circles. Two separate parameter values each were obtained from regression relationships for the accident, therapy patient, and combined data. Compared to the accident regression line, the therapy line indicates a somewhat earlier onset for the same doses. This likely reflects the propensity for emesis; whereby the patient undergoing total body irradiation (TBI) was moved (revolved 180°) for bilateral exposure protocols, a frequent practice in the earlier days of TBI therapy for cancer patients.
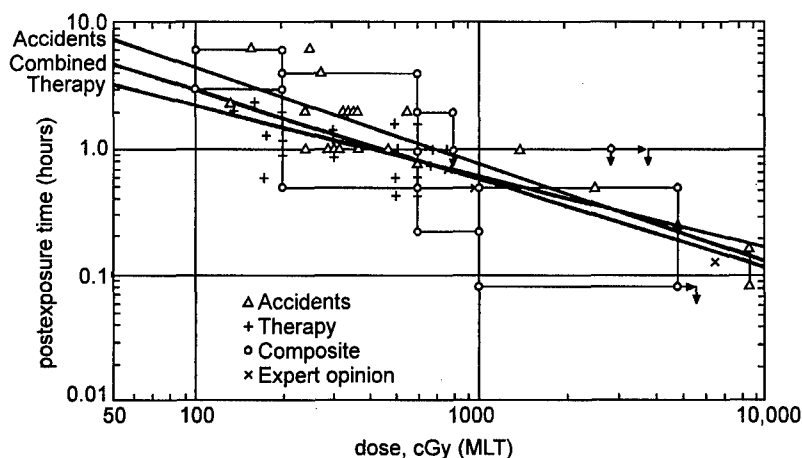
Fig. 8. Onset of vomiting related to dose.

Questionnaire response	Performance $P = (t_0/t)$	Linear/logistic regression					
no time increase $(t = t_0)$	1	$\log[P/(1-P)] =$					
		$\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6$					
time increase $(t_0 < 1 < \infty)$	>0 <1	NR	(UG)	(LG)	(FW)	(HY)	(IB) (FL)
could not perform task $(t = \infty)$	0	GB HD	(UG) (SK)	(LG) (SY)	(MU) (RD)	(OC) (OD)	(RE) (ME)

$$P = \left\{ 1 + \exp\left[- \left(\beta_0 + \sum_{k=1}^{k} \beta_k X_k \right) \right] \right\}^{-1}, \ X_k = (1,5), \ 0 \leq P \leq 1$$

Fig. 9. Data analysis - task performance regression.

Performance Degradation Algorithm

The development of the algorithm for radiation-degraded combat performance and the supporting data are documented in the technical reports [5,6]. The metric to gauge the level of degraded performance is related to the time required to perform a combat task or specific activity. Also, the nature of the tasks considered are those that can be completed in a short time span (seconds to minutes) under normal conditions and, therefore, do not correspond to measures of endurance. Specifically, performance level is the ratio of the normal time to perform a given task to the time lengthened by stress, in this case radiation-induced effects (fig. 9). The type of response data obtained from the questionnaires administered to the SME groups are indicated in the first column of the chart. The second column indicates the corresponding numerical value, and the third column gives the linear/logistic relationship used for the multiple regression analysis of the data. Given the P-values (performance data) and X-values (numerical value of the S/S severity) for the six S/S categories, the beta-parameter values are determined for the nuclear radiation (NR) stressor (GB and HD are for chemical agents sarin and mustard, respectively).

From the regression relationship, the performance level is given by the logistic form shown on the chart. The logistic form was chosen to express performance in order to restrict the values in the interval (0,1) as given by the solid line plot (fig. 10). With a straight linear regression
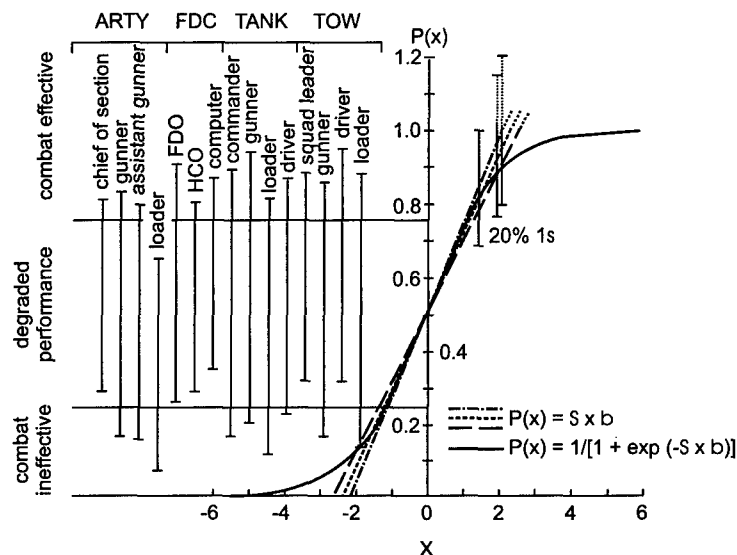

Fig. 10. Logistic vs. linear model for combat performance.

relationship, performance would not be restricted in the internal (0,1) as indicated by the broken line plots.

Performance Confidence Limits

Using the logistic performance function, the mean value of performance can be obtained by specifying any particular set of six severity-level values (Xn) for the S/S categories. Of course, any combination of values selected must conform to one of the S/S complexes found in the symptomatology map to properly relate dose and time postexposure to performance level.

The confidence bounds for performance level vary as a function of S/S complex, and generally increase with symptom severity, and hence with dose. They reflect the uncertainty in the SME estimates of performance level based on how the SMEs viewed task performance vis-a-vis ARS according to the S/S descriptions.

There are two kinds of confidence limits calculated from the SME response data (see fig. 11 and fig. 12). The plots are performance level (P) versus the complement of performance (1-P) for the predicted mean performance obtained with the performance algorithm. The predicted values lie along the diagonal line associated with the S/S complexes located along the abscissa shown by the short vertical tick marks. The corresponding response data from the questionnaires for each S/S complex along the abscissa are designated by asterisks. This means of plotting the predicted performance and the data are for convenience of comparison.

The solid error bars are the 95% confidence limits of the prediction. The dashed or dotted error bars are the 95% confidence limits of the forecast. The confidence limits for the prediction are a measure of the uncertainty in the regression line. The forecast confidence limits are a measure of uncertainty regarding the data, i.e., any similar additional data would be expected to lie within the outermost bounds with a 95% confidence level.
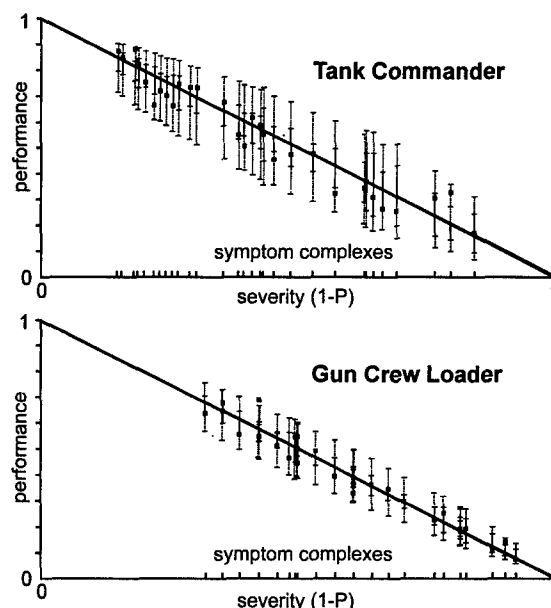

Fig. 11. Individual performance prediction, tank commander and gun-crew loader.

The predictions with 95% confidence are of primary interest. Based on the IDP data for mobile, ground combat units, average values range from 5 to 40% of the mean for, respectively, high and low severity of ARS, and hence, high and low dose extremes. In the mid-dose range (medium ARS severity) 95% confidence limits are approximately 10% of the mean performance. The corresponding mean
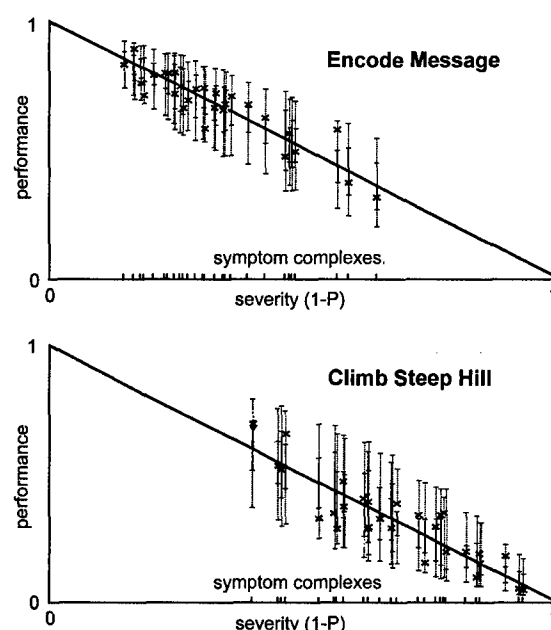

Fig. 12. Performance prediction after radiation exposure for dismounted infantry.
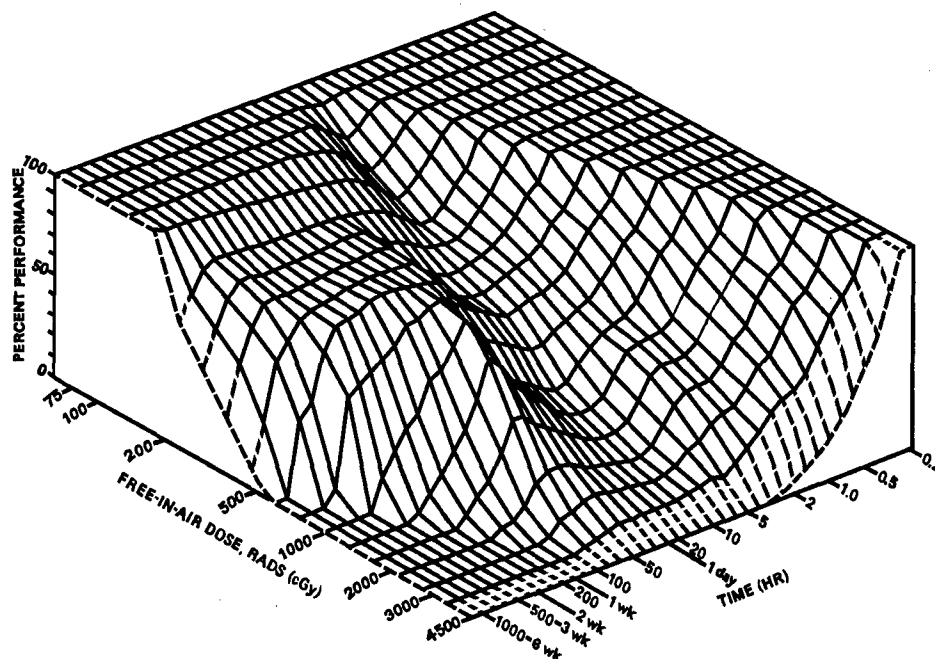
Fig. 13. Individual performance surface: tank commander.

values for dismounted infantry activities range from 9 to 40% for, respectively, low and high ARS severity, with a value of about 25% for medium severity (and dosage).

Estimates of the 95% confidence limits for the actual severity would require propagating the uncertainties through all stages of the analysis, including dosimetry, S/S incidence, severity level, and performance, as indicated above. Unfortunately, such data for either dose or S/S severity are not available. However, assuming that the IDP dose boundaries represent the 95% confidence limits for convenience of illustration, and utilizing the nausea and vomiting 95% confidence limits based on the probit analysis, 95% confidence limits approximately 35 to 60% larger and smaller than the mean degraded performance might be expected.

The charts also illustrate how ARS is perceived to affect the performance of two different kinds of combat crewmembers. The gun-crew loader performance is degraded to a greater extent than that of the tank commander. This is expected considering that the former activity demands greater physical exertion than the latter. The same can be said comparing two different kinds of combat activities common to a foot soldier—

"climb a steep hill" and "encode a message" (fig. 12).

Mapping the mean predicted performance functions onto the dose x time symptomatology map gives a three-dimensional picture of the degraded performance surface, as illustrated for combat activities performed by a battle-tank commander during a brief engagement scenario (fig. 13.). The 95% confidence limits based on performance can also be illustrated for the artillery loader using the RIPD code as illustrated in three charts (see figures 14,
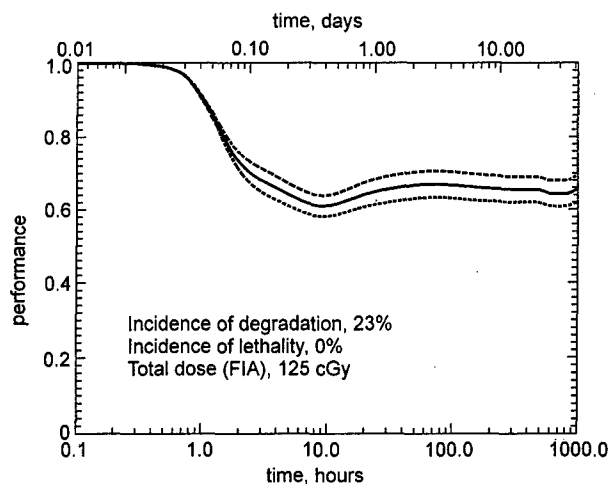

Incidence of degradation, 23%
Incidence of lethality, 0%
Total dose (FIA), 125 cGy

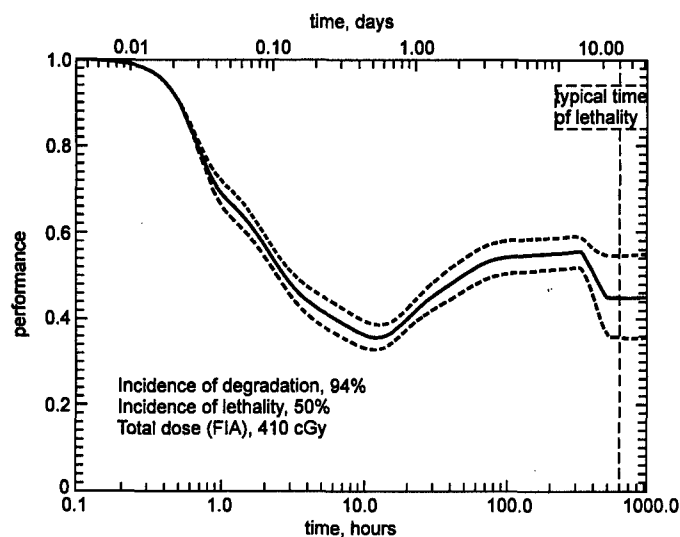**Fig. 14.** 125 cGy prompt dose: artillery loader.

Fig. 15. 410 cGy prompt dose: artillery loader.

15, and 16.) The plots in charts which show the 95% confidence limits represent a cut in a 3D dose x time plane along the time axis.

Efficacy of Antiemetics

The efficacy of introducing antiemetics (Ondansetron and Granisetron) in terms of performance level can be illustrated using the RIPD code calculations where antiemetics are assumed to be 100% effective contrasted with the absence of an antiemetic. First, one of the effects of antiemetic use can be gleaned from the dose × time symptomatology

map. Figure 3 shows S/S complexes that are shaded along the leading edge to illustrate the regions that would be most affected. The S/S complexes shaded black would be devoid of any severity greater than unity, and hence the "normal" state. Those to the right that are shaded gray would also represent a fairly benign state of ARS initially. As the ARS progresses with time, S/S categories other than UG distress would increase, although with the early UG effects being absent.

The effect of completely eliminating UG distress on tank-commander performance, through the use of a 100% effective antiemetic, would be dramatic, even
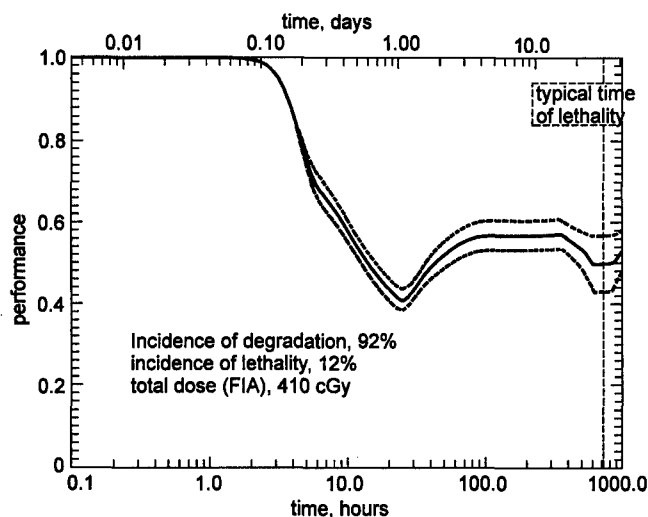

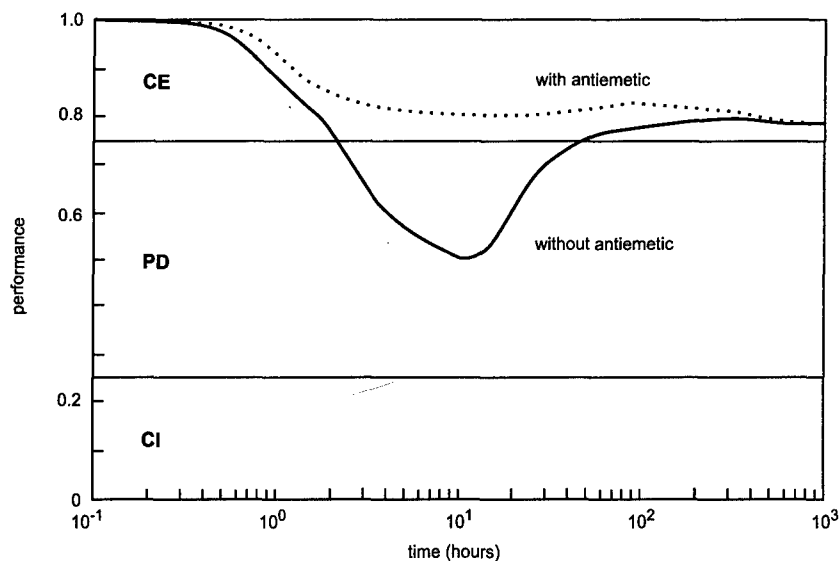**Fig. 16.** 410 cGy total dose over 24 hours: artillery loader.

Fig. 17. Tank commander dose = 410 cGy.

though exposed to a 410 cGy FIA dose (fig. 17). The dotted line is the time profile *with* the antiemetic and the solid line is the time profile *without* the antiemetic. The personnel risk and casualty criteria (PRCC) designations developed by the U.S. Army Nuclear and Chemical Agency (USANCA) are also indicated and have the following meanings:

- CE (combat effective): 75 to 100% fraction of capability

- PD (performance degraded): 25 to 75% fraction of capability

- CI (combat ineffective): 0 to 25% fraction of capability.

The tank commander remains CE with an antiemetic that would eliminate prodromal nausea and vomiting. However, for a more strenuous activity and the same dose (410 cGy), even with the total curtailment of nausea and vomiting, performance drops to the PD category since other debilitating effects such as FW are still present (fig. 18).

Another way of illustrating the relative effect of antiemetic administration is to specify that the
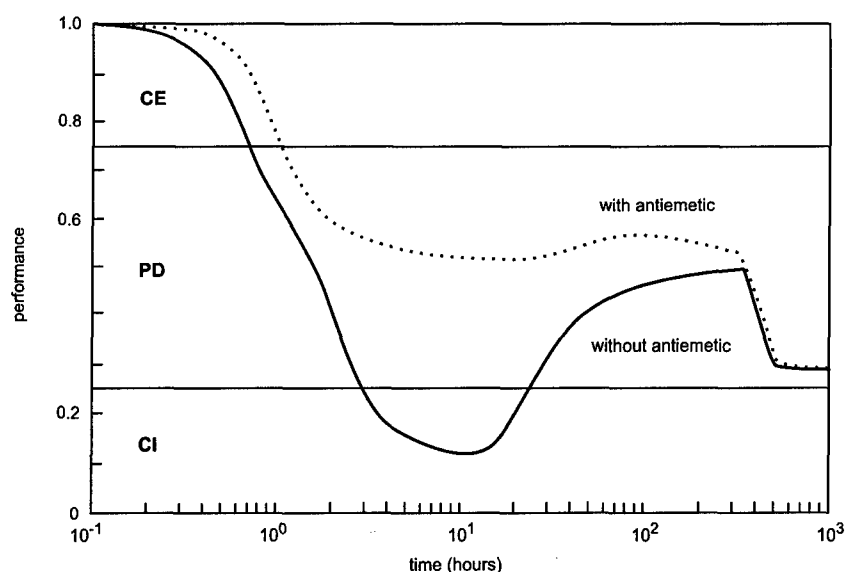

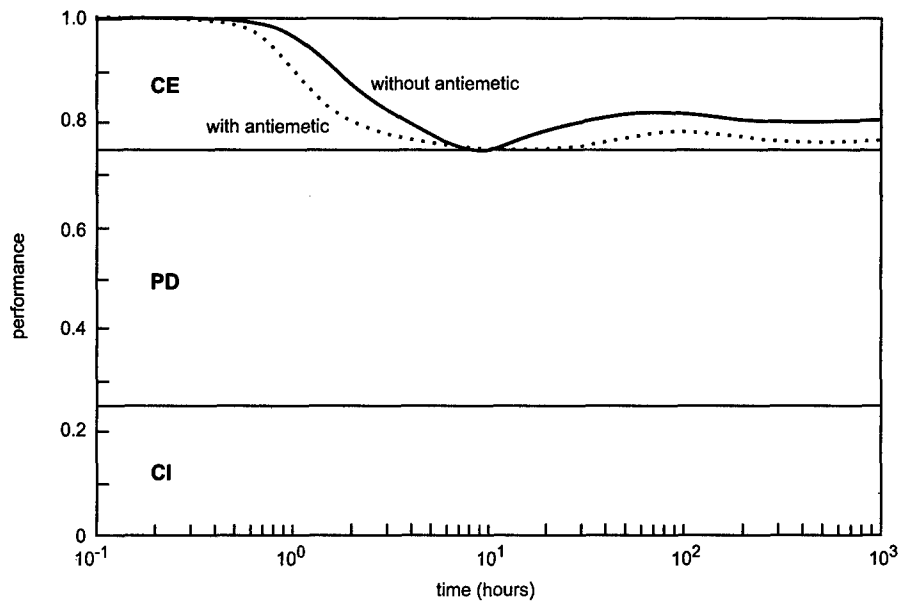**Fig. 18.** Infantry task: climb a steep hill; dose = 410 cGy.

Fig. 19. M60A3 tank loader—back calculation for performance = 0.75%.

fraction of remaining performance never drops below the CE range, i.e., 75%. The effect in terms of the difference in corresponding dose level *with* and *without* antiemetics is given for two different crew-members from two different combat crews engaged in their respective activities (fig. 19 and fig. 20). The results of the RIPD calculations indicate factors of 2.4 and 3.1 in the dose ratios *with* and *without* antiemetics for, respectively, the two crew-members.

Still another way to illustrate the relative effect of antiemetic administration is to consider the lowest level of casualty definition, also according to the PRCC developed by the USANCA, given as follows.

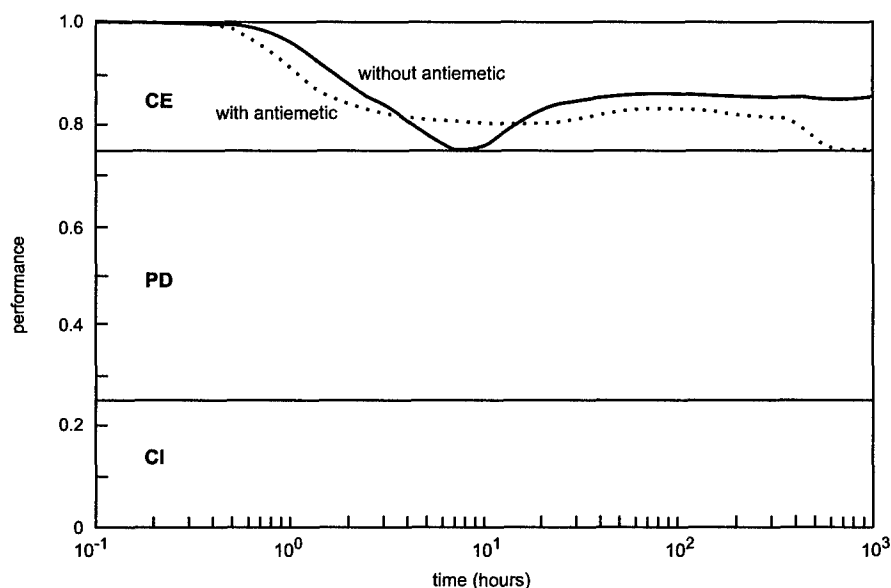Latent Ineffectiveness (LI): PD within 3 hours which is sustained for at least 6 weeks or CI at any time

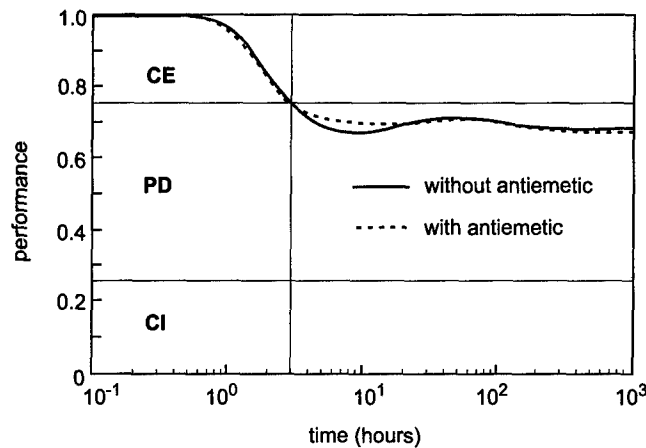**Fig. 20.** Tank commander—back calculation for performance = 0.75%.

Fig. 21. Artillery loader—back calculation for latent ineffectiveness. Without antiemetic, dose = 81.10 cGy; with antiemetic, dose = 93.5 cGy.

The performance of the artillery loader of the 155 mm SP Howitzer who handles a 100-lb. projectile, is not much different *with* or *without* an antiemetic, where the doses differ by only 12 to 13% (fig. 21). This is primarily due to the radiation-induced FW (RIFW) that also occurs when exposed to ionizing radiation. Quite a different performance profile is given for the tank commander for conditions of LI with antiemetics. Tank commander performance doesn't fall below the CE category until about 100 hours postexposure; whereas, without antiemetics, the performance recedes to the PD level between 1 and 2 hours postexposure and remains that way (fig. 22). The performance level with antiemetics remains below that without antiemetics in the PD region, owing to the somewhat larger dose in the former case.

Conclusions

Degraded performance from nuclear irradiation insult is derived directly from the S/Ss of ARS that are divided into six categories, each based on anatomical and physiological considerations and how performance capability would be expected to be affected. Performance level is mapped from S/S complexes as a function of dose and time postexposure based on a logistic algorithm. The algorithm provides fractional performance level according to the relative length of time to complete tasks or combat activities.

The 95% confidence limits placed on predicted performance level are derived from the performance data obtained from questionnaire responses provided by
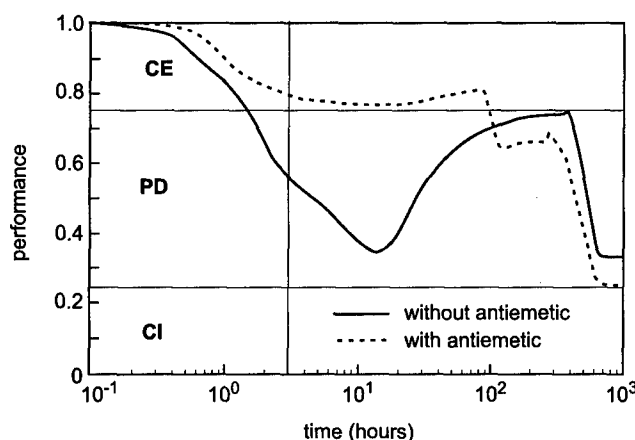

Fig. 22. Tank commander—latent ineffectiveness. Without antiemetic, dose = 676.29 cGy; with antiemetic, dose = 769.43 cGy.

SMEs queried regarding the projected capability if suffering the effects of ARS. Uncertainty in projected performance level that would also include the propagation of error from data such as dosimetry and S/S response are not available to perform the appropriate calculations. However, rough estimates indicate 95% confidence limits somewhere in the 35 to 60% range above and below the mean.

Since nausea and vomiting are among the most important S/Ss that could compromise performance, a considerable amount of effort has been directed toward their amelioration through effective antiemetics. Using the RIPD code, calculations were performed that illustrate the dramatic effects that can be realized in ameliorating the effects of nausea and vomiting under ideal conditions. Nausea and vomiting could very well serve as partial indicators of the magnitude of dose for individuals exposed to radiation, particularly in terms of onset time, especially when combined with other responses, namely when the exposure is prompt. However, if antiemetics are administered, the nausea and vomiting responses would make biodosimetry application ineffectual.

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Appendix D

Session IV

NATO Perspectives on Biological Indicators of Radiation Exposure

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Assessment of the applicability and accuracy of biological changes induced by ionizing radiation to determine which (alone or in combination) will quickly provide the best evaluation of actual damage sustained by individuals will enable medical officers to choose the most appropriate treatment at the earliest time. This ability will enhance recovery, operational effectiveness, and survivability. This assessment has been the focus of the Biological/ Clinical Indicators Subgroup of NATO RSG.23/ Panel VIII.

An overview of biological and biophysical techniques to assess radiation exposure has recently been published by Greenstock and Trivedi [1] and is discussed elsewhere. The NATO subgroup's program examines the developments of several indicators. It should be noted that parameters which have been under study previously, and which might be useful as biological indicators are not applied any longer (e.g., monoamineoxidase activity decrease in serum, prostaglandin release (urine), volatile exhaled hydrocarbons, and histamine release).

Research on biological indicators has been concentrated on whole-body irradiation and partial-body (heterogeneous) irradiation. For whole-body irradiation, the hematological parameters include: a) changes in cell populations, b) effect of dextran sulfate, and c) immune cell abnormalities. The cytological parameters include: a) micronuclei, chromosomal aberrations (dicentrics), and b) extra chromosomal breaks as detected after premature chromosome condensation. The detection of DNA damage after whole-body irradiation has involved the immunochemical detection of radiation-induced DNA breaks in peripheral white blood cells.

The biological indicator to determine partial-body (heterogeneous) irradiation has relied on the dose-dependent decrease in the nuclear area in skin keratinocytes.

Whole-Body Irradiation

Dextran Sulfate Mobilized Reserve Cells. Biological dosimetry can be based on the decrease in the capacity of dextran sulfate to mobilize reserve cells in the peripheral blood. So far these observations have been made only in mice. Human reserve cells can also be mobilized by dextran sulfate. A radiation dose dependence in humans has not been studied as yet.

Immunological-Sensitivity Score. Combining the decrease of subsets of B-lymphocytes and T-suppressor cells post-irradiation, and comparing this to the respiratory burst response before irradiation, will indicate individual radiobiological response to irradiation and eventual outcome (prognosis).

Micronucleus System. The micronucleus system shows promise. Practical application on a large number of samples within a short time frame (hours) is problematic. Literature data indicate that micronuclei in white blood cells show a large inter- and intra-individual variation in both background levels and radiosensitivity. This results in the problematic detection of radiation doses smaller than 0.2 Gy, and an uncertainty in the detection of a radiation dose of at least 40%. Recent results suggest that at least in cultured exponentially growing cells, the sum of the number of cells containing micronuclei *plus* the number of apoptotic cells is a better parameter for

determining radiation dose than counting micronuclei alone.

Detection of DNA Damage. Currently, the immunochemical detection of DNA damage appears to be a method that can be applied under simple conditions, provided the required instruments are present [2]. It is a simple, fast immunochemical assay which can be carried out on whole blood and gives results concerning radiation dose within 1.5 hours after blood sampling (finger puncture or arm puncture). In addition, several samples can be analyzed simultaneously. A dose-dependent increase of single-strandedness in the DNA of irradiated human white blood cells (*in vivo* and *in vitro*) is observed. Immediately after irradiation, 0.2 Gy is the lower detection limit. As a result of fast repair, 1 hour after exposure the lower detection limit will be about 2 Gy. Analysis can be carried out for up to 6 hours after exposure if blood is collected in a repair inhibiting solution at ambient temperature within 1 hour after exposure.

In an intercomparison dosimetry project also intended for calibration of physical dosimeters, this biological method was tested after blood was exposed in a realistic radiation field. Moreover, the assay can be set up quickly and carried out successfully in a more simple environment. For the further validation of this method, inter- and intra-individual variation still has to be studied, both after *in vitro* and *in vivo* irradiation of human blood.

Chromosomal Aberrations. Cytological dosimetry appeared to be a valuable supplement to physical dosimetry in the case of inadvertent radiation exposures. Cytological analysis of peripheral blood lymphocytes is preferred since these have the advantage of being easily obtainable and changes induced by irradiation are of the chromosome type, involving both chromatids equally. Structural chromosomal aberrations are induced *in vitro* and *in vivo* to approximately the same extent; therefore, reference dose-effect calibration curves can be easily examined for different radiation qualities. The induced aberrations can be examined for a long time after radiation exposure since the half-life of the blood lymphocytes is approximately 3 years.

Inter-individual variations in the background levels of unexposed samples render estimates below 0.05 Gy almost unattainable with any realistic degree of reproducibility. The main difficulty in using aberrations is the great labor needed to get reasonable confidence limits on the results. In addition, data are available only 2–3 days after blood sampling. Important progress has been made making use of premature chromosome condensation [3], hybridization probes and automation, i.e., making use of a metaphase-finder. Nevertheless, analysis of more than five samples a day is still problematic.

Partial-Body (Heterogeneous) Irradiation

A dose-dependent decrease of nuclear area (1-6 Gy) in human skin was observed at 24 hours both after *in vivo* and after *ex vivo* exposure. The same results were obtained after *in vivo* exposure of primate skin. This procedure is time consuming but can be conducted in a routine laboratory if the apparatus is present.

Combined Injuries, i.e., Irradiation With Other Injuries (e.g., Burns, Wounds)

Combined injuries represent a significant complication that must also be considered to achieve an accurate assessment of injury. Because biological indicators of radiation alone are still being developed, the presence of other injuries and stressors are complicating factors which remain to be studied.

Collaboration Between Dosimetrists and Biologists

Research collaboration between dosimetrists and biologists is indispensable to successfully establish and predict the value of measured doses. The objective is to determine quantitative links between physical dosimetry and specific biological damage which in turn determines an individual's acute or late

radiobiological response. It could be demonstrated that DNA damage in irradiated blood samples can correlate with physical dose both for neutron and gamma irradiations. This could also be demonstrated with respect to the induction of micronuclei and chromosomal aberrations.

Conclusions and Recommendations

For medical assessment, medical staff use various clinical indicators for determining the severity of injury both from radiation and other sources. Advice on these assessments, particularly for the radiation-exposure patient with other injuries and especially burns, is required. It is important to achieve a high degree of automation with multi-parametric analysis in any biological "dosimetry" system. This high degree of automation has been met in the development of the immunochemical assay for the detection of DNA damage, leading to much progress in the measurement of chromosomal aberrations.

The validation of the developed methods and their application for determining radiation exposure under field conditions has been an important goal, now realized. This would not have been possible without the cooperation and pooling of resources of the participating NATO defense laboratories.

Currently, there is no biological indicator available which can accurately detect low-level radiation exposure in personnel (between 0.05–70 cGy). As can be derived from mutation-induction-studies, an accumulated dose of only 0.05 Gy will result in a 25% increase of single-exon deletions. To detect this increase, single-exon deletion mutants must be detectable in an environment of 10^6 unmodified cells.

A modified PCR-amplification method in which the DNA fragment containing the deletion is amplified may provide the required sensitivity. In preliminary experiments we could already detect plasmids containing a 160-bp deletion among a 10^6-fold extent of plasmids not containing the deletion.

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Radiation-Induced Apoptosis in Human Lymphocytes

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Abstract

Experimental evidence indicates that radiation-induced apoptosis in human lymphocytes has the kinetics, sensitivity, and reproducibility to be a potential biological dosimeter. Human lymphocytes were irradiated in culture and two assays were used to measure the frequency of radiation-induced apoptosis: *in situ* terminal deoxynucleotidyl transferase (TdT) assay and fluorescence analysis of DNA unwinding (FADU) assay. Induction of apoptosis in lymphocytes irradiated *in vitro* was proportional to dose and could be detected following exposures as low as 0.05 Gy. Lymphocytes from individual donors had reproducible dose responses. There was, however, variation between donors. A prior exposure of lymphocytes to small doses of

radiation sensitized cells to a subsequent radiation exposure. Hyperthermia initially sensitized then conferred resistance to radiation-induced apoptosis in lymphocytes.

The *in vivo* rate of appearance and longevity of radiation-induced apoptosis in human lymphocytes is unknown. We have determined, however, that mouse lymphocytes irradiated *in vivo* have a similar response to human lymphocytes irradiated *in vitro*. Apoptosis in mouse lymphocytes was measured using the comet assay. It was determined that samples could be incubated either as isolated lymphocytes or as whole blood. Preliminary data showed that apoptosis in lymphocytes could be measured after whole body irradiation of mice. Radiation-induced apoptosis was detectable provided lymphocytes were removed from the body within 1 hour postirradiation. Apoptosis was not detectable when lymphocytes were collected 24 hours postirradiation.

In summary, *in vitro* studies with human lymphocytes showed that radiation-induced apoptosis in lymphocytes was detectable at low doses, reproducible for individual donors, varied between individuals, and was modified by prior exposures to radiation and heat. *In vivo* studies with mice indicated that lymphocyte samples should be collected soon after whole body irradiation. In conclusion, we suggest that apoptosis in lymphocytes may be used to assess individual sensitivity to radiation and predict the biological consequences of a radiation exposure.

Introduction

In biological dosimetry it is difficult to measure the biological damage and the subsequent risks associated with radiation exposure. Chromosome aberrations and micronucleus formation are classic biological endpoints that have been used to assess radiation damage to a cell. Alternatively, we present new evidence indicating that death of white blood cells (apoptosis) may also be a useful biological indicator of radiation exposure.

Apoptosis is a form of cell death with distinctive morphological and biochemical characteristics.

Fragmentation of nuclear DNA is one of the biochemical events that occurs in apoptotic cells and distinguishes them from other cells and other modes of cell death. We have used three assays to measure radiation-induced apoptosis in peripheral blood lymphocytes from humans and mice: the *in situ* Terminal Deoxynucleotidyl Transferase (TdT) assay, Fluorescence Analysis of DNA Unwinding (FADU), and the comet assay. Here we report evidence that supports the idea that apoptosis of irradiated lymphocytes has potential as a short-term biological dosimeter, appropriate for accident scenarios, and also may be useful to assess individual radiosensitivity.

Methods

Human Cell Culture and Irradiation. Blood samples were collected from healthy male volunteers in heparinized tubes. Lymphocytes were isolated, washed in Hank's Solution, and were resuspended at 4.0×10^5 cells/ml in complete growth medium. Cells were irradiated at $37°C$ in culture medium with either x-rays or ^{60}Co γ-rays. The two assays used to measure DNA fragmentation associated with apoptosis in human cells were TdT and FADU [1,2].

Mouse Cell Culture and Irradiation. Male CBA mice were given 1.5 Gy whole body ^{60}Co γ-rays. Blood samples (10–20 μl) were obtained by orbital bleed or tail puncture. The blood was immediately diluted in 10X complete RPMI media and sealed in a sterile 1 ml Eppendorf tube. The tube was incubated for 24 hours and then assayed for apoptosis using the comet assay [3].

Results

Human Lymphocytes Irradiated In Vitro. Human lymphocytes undergo radiation-induced apoptosis in a time- and dose-dependent manner. Apoptotic cells were detectable after 6 hours of incubation following a 10-Gy exposure (fig. 1A). At 24 hours of incubation doses as low as 1.0 Gy could be detected above control levels (fig.1B). Radiation-induced apoptosis seemed to show a linear response up to 10 Gy (fig. 1B).
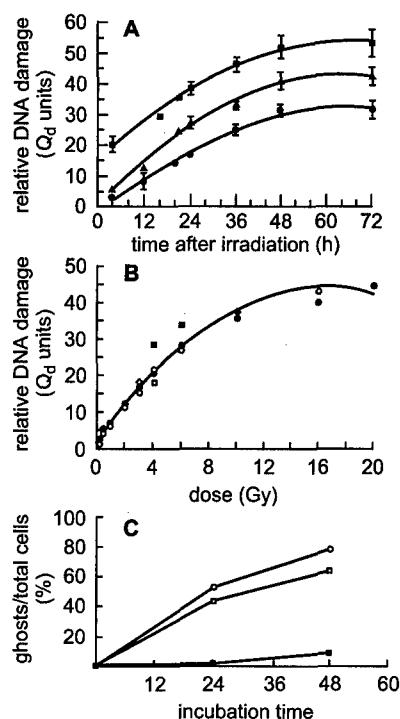
Fig. 1. (A) Time dependent appearence of apoptotic human lymphocytes following *in vitro* exposure to 3 (●), 6 (▲), and 10 (■) Gy of ^{60}Co γ-rays. (B) Dose response for radiation-induced apoptosis in human lymphocytes measured 24 hours after *in vitro* exposure. Different symbols represent data from different donors. Closed symbols indicate the same individual from repeat experiments. (C) Time dependent appearence of apoptotic mouse lymphocytes following *in vivo* irradiation (1.5 Gy ^{60}Co γ-rays) and 24 hours *in vitro* incubation (control mice [●,■], and irradiated mice [o,□]).

***Mouse Lymphocytes Irradiated* In Vivo *and Incubated* In Vitro.** Apoptotic lymphocytes could be detected in whole blood samples after 24 hours of incubation, provided the blood was removed from the mouse within 1 hour postirradiation (fig. 1C). Apoptosis was not detectable in lymphocytes from blood samples taken 24 hours postirradiation (data not shown).

Conclusions

In conclusion, *in vitro* studies with human lymphocytes showed that radiation-induced apoptosis in lymphocytes was detectable at low doses, reproducible for individual donors, varied between individuals, and was modified by prior exposures to radiation and heat. *In vivo* studies with mice indicated that lymphocyte samples should be collected soon after whole body irradiation. We propose that apoptosis in lymphocytes may be used to assess individual sensitivity to radiation and may predict the biological consequences of a radiation exposure.

Acknowledgement

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Halo-Comet Assay

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Abstract

A simple procedure known as the "halo-comet" assay offers an accurate determination of changes in DNA organization (supercoiling) in individual cells; and the results are known to be equivalent to cell

survival. When x-rays were used, this assay was sensitive in detecting 0.5 Gy-induced changes of DNA organization prior to the damage repair, and 2.0 Gy-induced changes after the damage repair. This assay was also useful for protracted exposures to x-rays. When the image processing is automated, this assay could be a potential candidate for a field-capable means of dose estimation.

Introduction

The "halo-comet" assay is an extensively modified modern version of the fluorescence "halo" assay. The "halo" assay was originally described by Vinograd et al. [1] and refined by Roti-Roti et al. [2]. This assay quantifies the alterations of DNA organization (supercoiling) in individual cells; the results are known to be equivalent to cell survival [3]. Unfortunately, this assay has a limitation in its sensitivity. For example, this assay can detect changes in DNA organization at radiation doses on the order of 2 Gy prior to the damage repair [2]. However, when the damage is repaired, the assay becomes insensitive below 10 Gy [2, 4]. In order to increase the sensitivity, our laboratory has been involved in the modification of the "halo" assay, and has established the "halo-comet" assay, which can detect small amounts of damage in DNA organization even after the process of damage repair.

Materials and Methods

Two different cell lines were used. HL-60 human lymphoma cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (GIBCO). MCF-7 human mammary carcinoma cells were maintained in IMEM medium supplemented with 10% fetal bovine serum, 10% HEPES, and zinc (GIBCO). Both cell lines were exponentially grown at the time of the experiments. A Seifert x-ray unit (Rich. Seifert & Co.) operated at 250 kVp, 15 mA with 0.5 mm copper plus 1.0 mm aluminum filtration was used to deliver radiation to the cells at a dose rate of approximately 2 Gy/min.

In order to produce the "halo-comet" structure, MCF-7 cells were dispersed into single cells with a trypsin solution. .These dispersed MCF-7 cells, or HL-60 cells in suspension, were resuspended in a concentration of 10^5/ml in ice-cold PBS, and mixed with the same volume of a 2% agarose solution (Sigma, type I) that was dissolved in PBS and warmed at 60°C. The mixture was quickly spread onto a slide glass, allowed to form into a gel (less than a minute at room temperature), and stored in ice-cold PBS. Then the cells in the gel were exposed to a dye-lysis solution: a mixture of 2 mM Tris, 0.5% Triton X-100, 2 M NaCl and 10 mM EDTA (TTNE) with twice the desired propidium iodide (PI) concentration. Cell lysis was carried out in the dark at room temperature, and the lysis time was 15–30 min. Then the gel, after cell lysis, was washed in distilled water for 1h and subjected to electrophoresis in a TAE buffer (40 mM Tris, 20 mM acetic acid, and 5 mM EDTA). The time of electrophoretic separation was 20–30 min (3V/cm, 17 mA).

To visualize the "halo-comet" structure, the PI-stained DNA was excited at 545 nm; and the emission at 580 nm was observed with the aid of a 590 nm barrier filter under a reflected fluorescence microscope (Olympus, BH-2). The intensity of fluorescence light was enhanced using the Dark Invader Night Vision System (Meyers & Co. Inc.). The image was stored on video or digitized and processed with the use of an image processing software, Accuware (Automatic Visual Inspection).

Results and Discussion

In the modification of the existing "halo" assay, we first tried to produce the "halo" structure in an agarose gel, as opposed to producing it in a buffer solution, as described by Roti-Roti et al. [2]. This attempt was successful. We were able to observe the "halo" structure in an agarose gel (fig. 2, upper image). Since the structure was formed in an agarose gel, we tried applying an electric current to the gel, hoping that the DNA loops would be pulled from the "halo" structure. Following numerous attempts using different milliamperes and run times, the DNA loops were finally pulled toward the cathode.

The DNA loops were stretched and formed a tail (fig. 2, middle image). We prefer calling this tailed structure a "halo-comet" to distinguish it from the common names previously described as "halo" by Roti-Roti et al. [2], and "comet" by Olive et al. [5]. This "halo-comet" structure was indeed different from the alkaline "comet" structure (fig. 2, lower image), which was produced according to Olive et al. [5]. In the alkaline "comet" structure, there was a discontinuous distribution of fluorescence intensity from head to tail (fig. 2, lower image), suggesting that fragmented DNA strands were separated (tail) and removed from the nuclear matrix (head). In this assay, DNA damage (single strand breaks) is quantified in terms of a "tail-moment," a product of the amount of DNA removed from the head and the distance of migration [5]. In contrast to the "comet"

image, the "halo-comet" image showed a continuous fluorescence distribution (fig. 2, middle image), implying that DNA loops were still attached to the nuclear matrix and were stretched from the matrix by electric charges. Since no head and tail was distinguishable, it was impractical to quantify the "halo-comet" image in terms of a "tail-moment." Therefore, the longest axis (image length) or pixel numbers (image area) occupied by the "halo-comet" image were determined to quantify the damage of DNA organization.

To optimize the assay, the concentration of PI was varied (0–50 µg/ml). When control (0 Gy) cells were used, the "halo-comet" structure became larger with increasing PI concentration. The largest structure was maintained when the PI was higher than 10 µg/ml. For irradiated (20 Gy) cells, the largest structure was observed when the PI was higher than 30 µg/ml. Since a maximal difference between the control and the irradiated cells was observed at 30 µg/ml, this PI concentration was chosen as a standard. When HL-60 and MCF-7 cells were irradiated (0–20 Gy) and subjected to the "halo-comet" assay, the amount of DNA pulled from nucleoids (nuclei remaining after removal of proteins) increased linearly with increasing radiation doses up to 6 Gy (data are not shown). The sensitivity of the "halo-comet" assay was found to be far greater than that of the existing "halo" assay. For instance, the changes in DNA organization following doses as low as 0.5 Gy prior to the damage repair were detected by the "halo-comet" assay, which is a significant advancement over the existing "halo" assay. For the "halo-comet" assay, differences between the 0 Gy versus 0.5 Gy-induced damage was statistically significant ($p < 0.05$, Student-t test), either when analyzed for image area by 50 samples or when analyzed for image length by 25 samples (data are not shown).

Since the "halo-comet" assay can detect 0.5 Gy-induced alterations of DNA organization, we attempted the detection of residual alterations of DNA organization remaining after the repair processes. MCF-7 cells were exposed to 0, 2, and 4 Gy of x-rays and were incubated at 37°C for 30 min. These cells were then subjected to a gel formation and to the "halo-comet" assay. The resultant "halo-
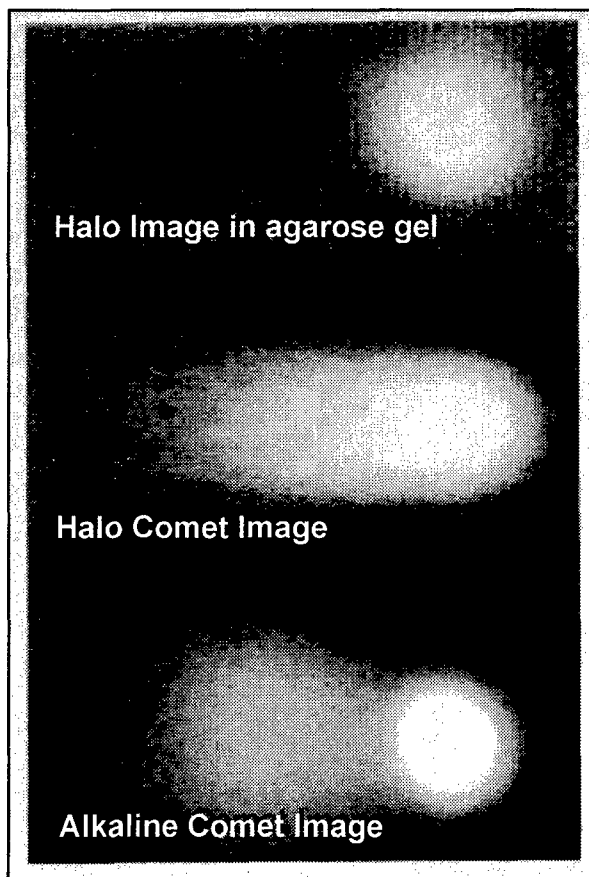

Fig. 2. Comparison of different images of HL-60 cells. The "halo" image was formed in an agarose gel (top). The "halo" structure was stretched by electrophoresis to form a "halo-comet" image (middle). An alkaline "comet" image (bottom) was quite different from the "halo-comet" image (middle).
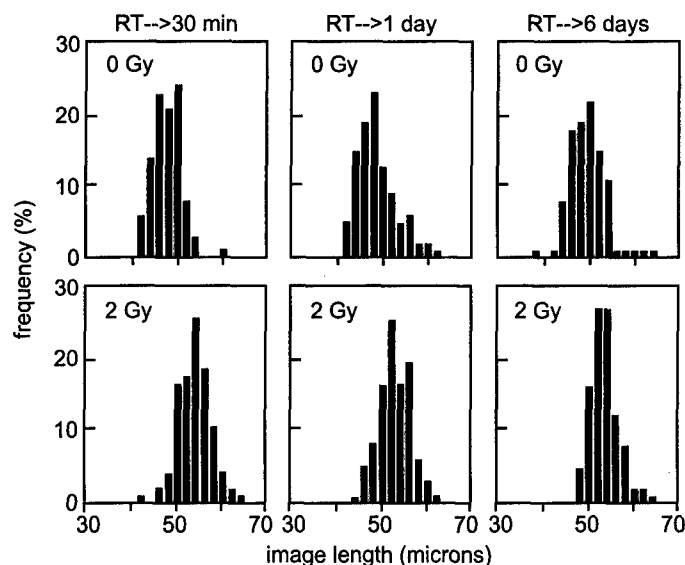
Fig. 3. Residual damage remained after the repair processes. MCF-7 cells were exposed to x-rays and incubated for 30 min. These cells were then subjected to the "halo-comet" assay by employing two image parameters. For each group, 100 images were analyzed.

comet" images were analyzed in terms of both image length and area; the results are shown in fig. 3. For both image length and area, a distinction between the control and 4 Gy-irradiated cells appeared to be clear; so only the paired data sets for 0 versus 2 Gy data were subjected to the Student-t test. As shown in table 1, the difference between 0 and 2 Gy data was highly significant (p < 0.0004) with samples as small as 25 for both endpoints. This sensitivity is superior to the sensitivity of the "halo" assay, which can only detect a difference between 0 and 10 Gy of DNA damage after the repair processes [4]. When HL-60 cells were used, the "halo-comet" assay was able to detect 2 Gy-induced alterations of DNA organization even 1 or 6 days after the irradiation, as shown in fig. 4, where the endpoint of the assay was image length.

Table 1. Student t-test (p values) for the changes in DNA organization induced by 0 versus 2 Gy of X-rays. "Halo-comet" assay was performed after completion of the repair processes.

Sample size	Image area	Image length
25	0.000024	1.2×10^{-12}
50	9.9×10^{-10}	$< 10^{-16}$
100	0.000333	$< 10^{-16}$

Since residual alterations of DNA organization (2 Gy) remained after repair processes were detectable (figs. 3 and 4), we attempted to study the effect of a
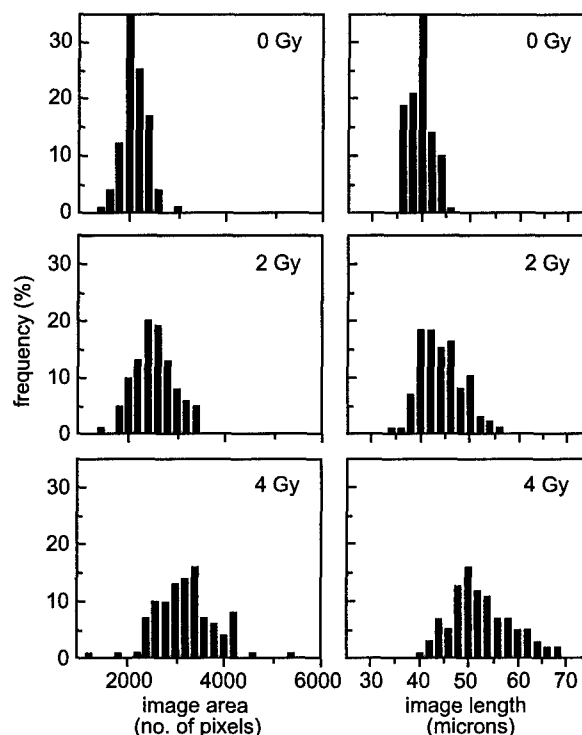

Fig. 4. Residual damage remained even after 6 days of recovery. HL-60 cells were exposed to x-rays and incubated for up to 6 days before the "halo-comet" assay. The image was analyzed in terms of image length; 100 images were analyzed for each group.

protracted exposure to x-rays. MCF-7 cells were exposed to daily 2 Gy doses for 3 days (Monday, Tuesday, and Wednesday). The changes in DNA organization were determined on the following Monday. As shown in fig. 5, the amount of damage increased with increasing number of exposures or increasing total doses, when determined by image area. Although the assay was carried out 5 days after the completion of daily exposures, dose-dependent alterations of DNA organization were clearly observed.

As demonstrated in the present report, the "halo-comet" assay has great potential as a field-capable means for dose estimation. This assay is based on a non-radioactive procedure, and is sensitive to 2 Gy-induced genotoxicity. The result of this assay is known to be equivalent to cell survival [3]. This assay can handle a large number of samples simultaneously. For example, processing 100 samples may not be difficult for trained personnel, since four

different samples can be loaded onto a slide glass; and 25 slides may be loaded for electrophoresis at the same time. Another advantage is the speed of the assay. The "halo-comet" images can be produced in less than 2 h for every set of sample loading. For dose estimation, these produced images need to be analyzed by using an image processor. When 100 images for each sample are to be analyzed, for example, a total of 10,000 images for 100 different samples must be analyzed. Therefore, automating the image processing is essential to shorten the time for dose estimations of a large volume of samples; the overall time required for dose estimations might depend on the success of the automation. Many variables that will affect the "halo-comet" assay have not been determined. Further studies are absolutely necessary to optimize the assay for a field-means of dose estimation.

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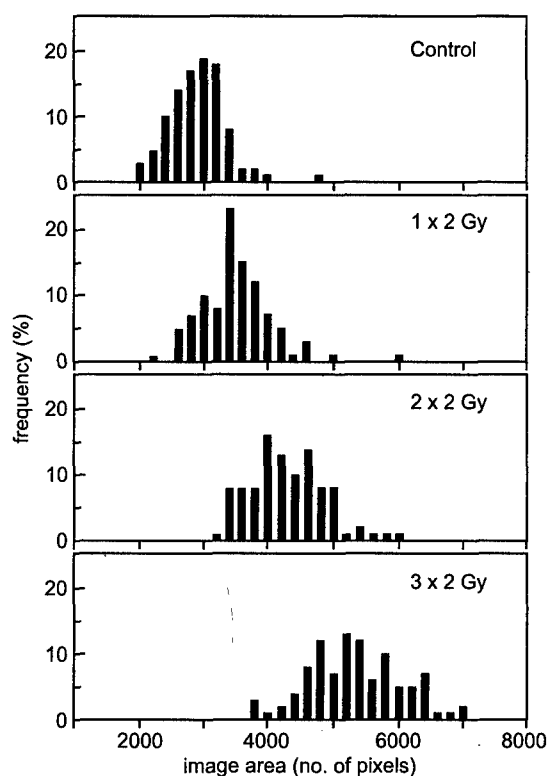
Fig. 5. Persistent residual damage remained after protracted exposures. MCF-7 cells were exposed to control, 1 x 2 Gy, 2 x 2 Gy, and 3 x 2 Gy of x-rays as described in test above. The "halo-comet" assay was carried out 5-7 days after irradiation. Residual damage remained after repair and was dose-dependent.

Radiation Damage in the Hematopoietic System

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Abstract

After exposure to ionizing radiation, even at sublethal doses, the cellularity and functional capacity of the immune system is compromised. This leads to increased susceptibility to disease and infection. The degree of impairment needs to be assessed. We have found that flow cytometry (FCA) is potentially one of the fastest, most accurate methods to achieve this. We have examined alterations in all of the major splenic and peripheral blood mononuclear cell populations in C57BL/6 mice following whole-body irradiation (0–700 cGy) to determine which cell populations may play a role in active immune suppression and/or hematopoietic recovery. A flow cytometric protocol has been established for the characterization and differentiation of the major mononuclear cell populations in the mouse spleen and whole-blood: T lymphocytes ($CD4^+$ and $CD8^+$ cells), B lymphocytes ($CD45^+$), natural killer (NK) cells, and monocytes/macrophages. Ionizing radiation caused decreased cellularity in both the splenic and whole-blood cells. In addition, the induction of apoptosis in the blood cells was assessed by the measurement of phosphotidylserine with the use of Annexin V. FCA revealed alterations in the relative composition of the constituent cell populations following irradiation, reflecting differential radiosensitivity, with selective enrichment of NK cells and $CD4^+$ T lymphocytes. Enrichment developed and persisted during the 7-day post-irradiation period. Some mononuclear cells became activated in a dose and time-dependent fashion following whole-body irradiation (WBI), as indicated by expression of CD71, the transferrin receptor. These cells were $CD34^+$ and $Thy1.2^+$ but were $CD4^-$ and $CD8^-$, as well as $CD45R^-$. The observed increase in NK cells corresponds to a previously reported increase in natural suppressor (NS) cells following total-lymphoid irradiation (TLI). The balance of recovery-inhibiting NK cells and recovery-enhancing $CD4^+$ T lymphocytes following irradiation may reflect or influence the degree of hematopoietic recovery, and may provide an indication of the extent of damage (biological dosimetry).

Introduction

Exposure to ionizing radiation can render the immune system incompetent due to systemic damage to the hematopoietic system. The ultimate survival of an animal following irradiation depends largely on the recovery of blood-cell formation. The hematopoietic capacity of the bone marrow, the organized lymphoid tissues, and individual small recirculating lymphocytes are all exquisitely radiosensitive. Since this system provides for renewal of mature circulating blood cells, those cells killed by irradiation or used up performing their functions will not be replaced until stem-cell recovery occurs. The recovery requires a sufficient number of surviving endogenous stem cells to proliferate, restore the stem cell pool, and provide specific progenitor cells which will differentiate into functionally mature cells. This process is regulated by a variety of cytokines which are produced by cells that constitute the hematopoietic microenvironment, including stromal elements and monocytoid accessory cells from peripheral blood [1-3]. The recovery of the hematopoietic system in mice given bone marrow transplants has been demonstrated to be enhanced by a distinct $Thy-1^+$ subpopulation resistant to radiation and cyclophosphamide [4]. The fact that such cells appeared to be relatively radioresistant and that a small population of radioresistant $Thy-1^+$ lymphocytes were found to survive *in vivo* in heavily irradiated animals [5] suggests that these cells may play a role in facilitating hematopoietic recovery. While the role of the $CD4^+$ T cell subset in hematoregulation has recently come under investigation [6-8], the relative radiosensitivities of the CD4 and CD8 subpopulations of T lymphocytes compared to other mononuclear cell populations have not been well characterized *in vivo* after irradiation.

This study was undertaken, using a murine model, to examine alterations in the major whole blood and

splenic mononuclear cell populations and their state of activation following whole-body irradiation (WBI). We employed flow cytometric analysis (FCA) using monoclonal antibodies to characterize and differentiate the various cell types by their specific and unique cell surface markers (CD), including CD71, the transferrin receptor [9, 10]. The induction of apoptosis was also determined by the measurement of Annexin V binding. The working hypothesis was that the relative concentration of certain cells is altered by irradiation, and this leads to the occurrence of immune system dysfunction or failure. It is our aim to identify particular alterations in relative cell number that might correlate with the dose received, the extent of resultant injury, and the immune-competent status of the animal.

Materials and Methods

C57BL/6 mice used in this study were female, weighed 18–20 g, and were obtained from Charles River (Montreal, Canada). The mice were housed in cages of five in the animal-care facility at the University of Ottawa, and given Purina Mouse Chow pellets and acidified water (pH=2.7) *ad libitum*.

Irradiation of mice was conducted in a [137]Cs GammaCell-40 shielded-drawer radiation source (Nordion, Kanata, ON, Canada) at a dose rate of 110 cGy/minute.

Whole blood samples were drawn by bleeding mice from the retro-orbital sinus, and prepared for flow cytometric analysis (FCA). Blood or spleen cells were stained with various combinations of fluorescently-labeled monoclonal antibodies or Annexin V -FITC and propidium iodide as listed in table 2. Samples were taken on days 1, 4, and 7 after exposure to WBI (25 to 700 cGy). Staining of blood and spleen samples was followed by lysis of contaminating erythrocytes and subsequent analysis by flow cytometry on a Coulter Epics XL flow cytometer (Coulter Electronics, Hialeah, FL).

Cell number was assessed by analysis on a Coulter-Zm Counter (Coulter Electronics). 20 µl of whole blood were diluted in 10 ml of Isoton II diluent (Coulter Electronics), treated with three drops of Zap-o-globin II (Coulter Electronics) for erythrocyte lysis.

Table 2. Panel of reagents employed for flow cytometric analysis.

Tube	Reagent	Cell marker
1	None	
2	Isotype controls	
3	Thy 1.2-PE CD4-FITC	T cells and CD4 T cell subset
4	Thy 1.2-PE CD8-FITC	T cells and CD8 T cell subset
5	CD45R-FITC	B cell
6	NK 1.1-PE	NK cell
7	MAC-FITC	Macrophage
8	Annexin V-FITC Propidium iodide	Apoptosis marker

Results

Blood drawn from mice on days 1, 4, or 7 after exposure to 100, 400, or 700 cGy WBI was stained according to the combinations of mAb in table 2, in order to identify numbers and percentages of individual peripheral blood mononuclear cell populations by flow cytometry. Figure 6 (a and b)
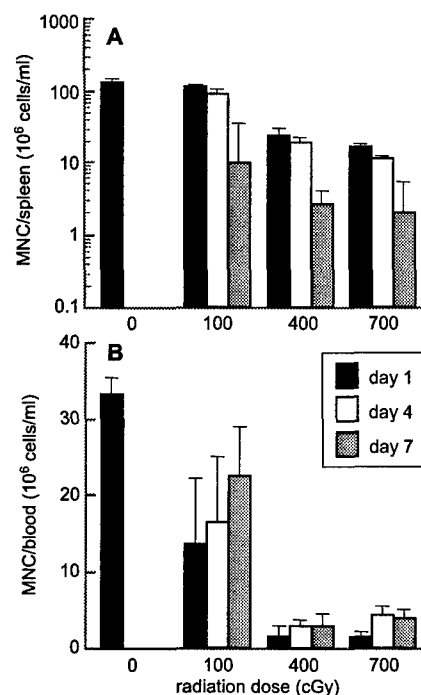

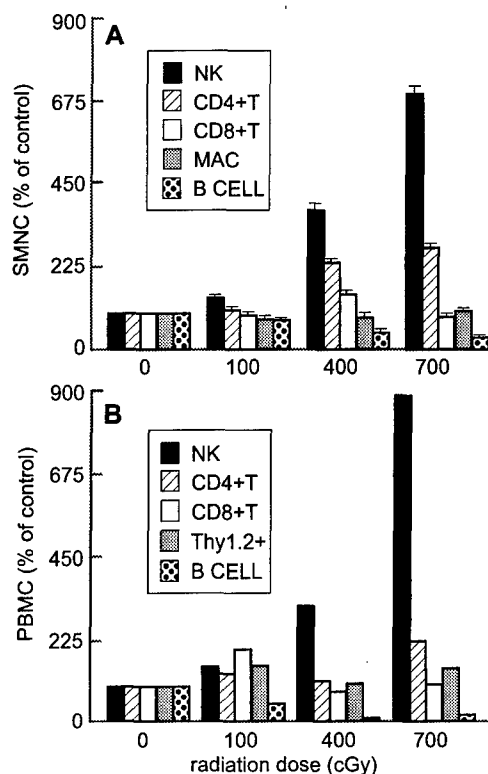**Fig. 6.** Effect of radiation on the cellularity of spleen and blood.

Fig. 7. Relative sensitivities of subpopulations of SMNC and PBMC to radiation.

Fig. 9. Kinetics of appearance of necrotic cells in the periphery.

demonstrates a dose-dependent decline in the total leukocyte count (splenic or whole blood) with increasing radiation dose.

Figures 7a and 7b show the alterations in the relative proportions of MNC populations on day 4 after radiation exposure, for the peripheral blood (PBMC) and spleen (SMNC), respectively. Clearly, in both compartments, B lymphocytes exhibit dramatic radiosensitivity; while NK and CD4$^+$ cells demonstrate marked radioresistance. Other MNC types
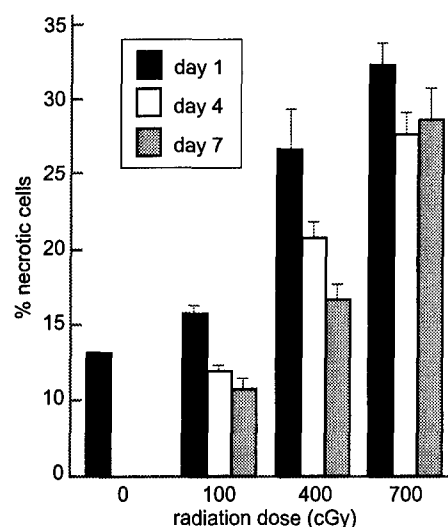
appear to show no statistically significant changes in their relative proportions after radiation exposure.

In figure 8, a population of cells morphologically distinct from normal blood MNC, as determined by flow cytometry, appears after radiation exposure. These cells are more granular than typical lymphocytes or monocytes, a feature characteristic of apoptotic cells. The prevalence of this population of cells is dose-dependent and declines with time, as shown in figure 9.

Figure 10 (a and b) demonstrates the radiation and time dependent Annexin-V response of the lymphocyte population. As early as 1 hour postirradiation, AnnexinV binding was observed with only 25 cGy. The more granular population observed in figure 9 was 100% for Annexin V and propidium iodide.
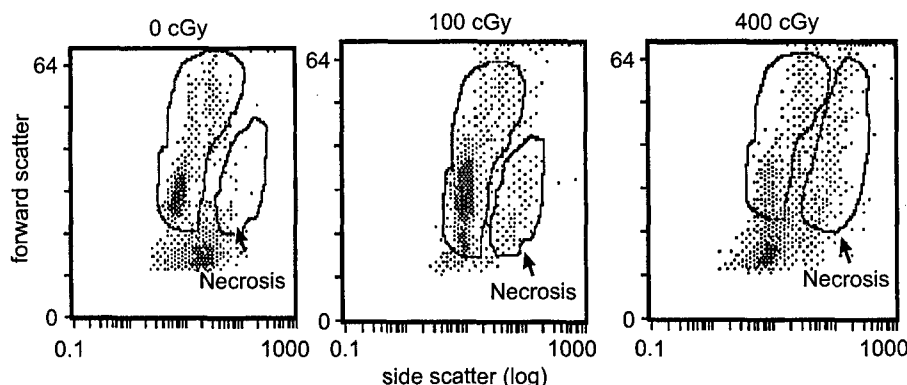

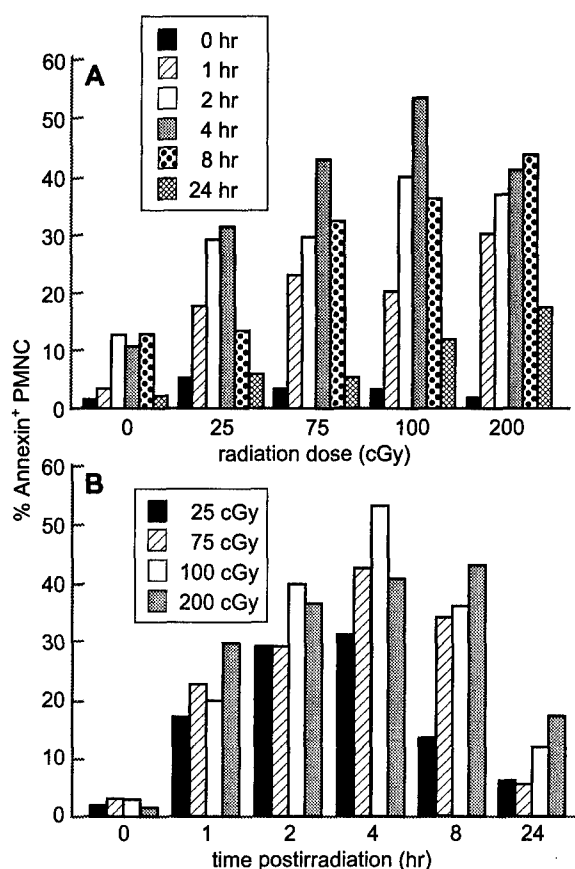**Fig. 8.** Changes in size (FS) and granularity (SS) with radiation dose.

Fig. 10. Annexin response of PBMC after irradiation.

This indicates that these cells were in the apoptotic or necrotic phase.

Tables 3 and 4 reflect the presence of CD71, the transferrin receptor; this marker can be found on cells that are differentiating. CD71 is found on a small proportion of splenic or peripheral blood leucocytes (3–4%) from unirradiated mice. All doses of radiation (100–700 cGy) failed to increase the percentage of positive cells on day 1 post-WBI. But higher levels of CD71-expressing cells were

Table 3. Percentage of CD71+ cells after whole body radiation.

Day	Control	100 cGy	400 cGy
1	1	3	3
4	3	9	3
7	3	11	2

Table 4. CD71 expression on cell subtypes.

Marker	% CD71$^+$
CD34	13
Thy1.2	13
CD4	0
CD8	0
CD45R	0
Macrophage	0
NK1.1	0

observed at different times and after different exposure doses (table 3). The population expressing the CD71 molecule was identified as CD34$^+$ or Thy1.2$^+$. Other markers were also tried but the CD71 cells were negative for these: CD4, CD8, B-cell marker CD45R/B220, macrophage marker F4/80$^+$, and NK1.1 (table 4).

Discussion

Suppressor-cell activation and severe damage to the bone marrow stem-cell population are two adverse effects resulting from exposure to ionizing radiation [11-13] which increases susceptibility to opportunistic infections. In the present study we have examined the relative radiosensitivity of SMNC and PBMC populations following whole-body irradiation. Such alterations may be indicative of a role for a particular subpopulation in active immune suppression or in hematopoietic recovery, and may be useful as an early biological marker for severity of damage to the critical immune system, and thus as an effective prognostic indicator for triage.

Whole-body irradiation of mice produced an exponential decline in cell survival of splenic cells and peripheral blood leukocytes in whole blood (fig. 6) as has been previously demonstrated both *in vitro* and *in vivo* [14]. The relative proportions of the mononuclear cell subsets in both organs dramatically

changed (figs. 7a and 7b) and were similarly affected. We examined changes in the major mononuclear cell populations in both organs including B lymphocytes, the CD4$^+$ and CD8$^+$ subsets of T lymphocytes, monocyte/macrophages and natural killer cells. Although all cell populations declined, they did so at different rates. In increasing order of radiosensitivity were: NK cells (D_0= 4.8Gy $\pm$ 0.8Gy), CD4$^+$ T cells (D_0= 3.1 $\pm$ 0.6), CD8$^+$ T cells (D_0= 2.2 $\pm$ 0.4), monocytes/macrophages (D_0= 1.7 $\pm$ 0.1), and B cells (D_0= 1.5 $\pm$ 0.1). The natural killer cells in this study were the most strikingly radioresistant cell population of the PBMC or SMNC. In control mice, NK cells made up only a small fraction on the total population (3%); whereas 7 days after 400 cGy irradiation, NK cells comprised almost one-quarter of the total PBMC or SMNC (results not shown).

The relatively high sublethal doses of radiation used in these studies induced hematopoietic depletion that was followed by vigorous recovery initiated from endogenous stem and progenitor cells that survived the irradiation [14]. *In vitro* studies have demonstrated that different lymphoid subsets have opposing effects on hematopoietic cell growth [15,16]. The hematopoietic recovery of an irradiated animal may then be influenced by the proportions of surviving recovery-enhancing and recovery-inhibiting cell types. T lymphocytes have been found to consist of two distinct subpopulations: a radioresistant population which can enhance hematopoietic recovery, and a radiosensitive population which can suppress it [4, 17]. However, the relationship of such subpopulations to the immunoregulatory CD4$^+$ helper and CD8$^+$ suppressor T lymphocytes has not been determined; but it has been recently reported that CD4$^+$ cells are stimulators of normal hematopoiesis and recovery following whole-body irradiation [7, 18]. The results reported here are in agreement with Williams et al. [7] in that the murine spleen contains a population of radioresistant CD4$^+$ T cells. As a result of the radioresistance of these cells, the spleens of irradiated mice became proportionately "enriched" with CD4$^+$ T cells. By 7 days post-700 cGy WBI, CD4$^+$ lymphocytes made up almost 50% of all SMNC, as compared to only 15% in control mice. The differing radiosensitivities of CD4$^+$ and CD8$^+$ T lymphocytes observed in the spleen are comparable to the radiosensitivities of the different types of T cells that exert helper and suppressor effects on hematopoietic recovery [17].

The type of cells expressing CD71 was demonstrated to be CD34$^+$ and Thy1.2$^+$; but they were not positive for CD4, CD8, CD45R/B220, or NK1.1. This suggests that CD71 is expressed on early progenitor cells and may be an indicator for early hematopoietic activity or possibly dedifferentiation (reactivation of dormant gene for CD71 expression, normally only functional during cell differentiation and development). CD34 and Thy1.2 account for 56% of the CD71 positive cells. The remaining cells may be other lineage specific cells. The absence of T cell subset markers (CD4 or CD8) also suggests that the CD71$^+$ cells are immature T cells that appear in the periphery after radiation or are somehow activated (19).

Our findings to date support the hypothesis that the relative decline in certain immune subpopulations is indicative of damage to and impairment of the immune system. For a biological "dosimeter," changes in proportion of cell types detected by flow cytometry represent a rapid way of assessing damage after radiation exposure and may also serve to assess the effectiveness of radioprotective agents in animal models or tissue culture.

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Automated Cytogenetic Assays in a Field Environment: Consideration of the Halo-Comet Assay

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Abstract

Cytogenetic-based bioindicator assays can provide diagnostically important information in forward-field military environments. In addition to assessing the effective equivalent biological dose, these assays provide estimates of the fraction of the body spared from radiation exposure. Based on its minimal pre-scoring preparation requirements, the halo-comet assay was identified as having potential for inclusion in a multi-assay system that can be used in the forward field. This paper's objective is to discuss the potential patient throughput of an automated halo-comet scoring system optimized for radiation biosimetry.

Radiation Biosimetry Requirements in the Forward Field

The following considerations were established to define candidate high-throughput automated cytogenetic assay systems relevant to field-level radiation biosimetry. Since sample preparation requirements generally limit their echelon I medical facility utility, cytogenetic-type assays are generally focused on echelon II and III facilities. These assays in military scenarios require significant per patient sampling and also require high patient throughput for radiation biosimetry triage. For processing in the triage mode, 20–50 cells per patient are required; in the dose assessment mode, 50–300 cells per patient are required. Typical patient throughput requirements range from 50–500 patients per day. In addition to minimized weight and footprint requirements, the field system must be simple to operate by non-specialist personnel.

Table 5 lists the existing cytogenetic assays which are potential candidates for inclusion in a radiation biosimetry multi-assay platform that can be fielded in echelon II and III medical facilities. Of the assays listed, the halo-comet assay exhibits both minimal pre-scoring cell processing properties and has the signal persistence sufficient to accommodate field use. The halo-comet assay does not require post-sampling incubation and is characterized by short processing time requirements. From a post-preparation scoring perspective, the comet and apoptosis assays can be considered as a subset of the halo-comet assay.

Halo-Comet Scoring

The halo-comet assay is based on nuclear suspensions in contrast to the normal comet assay that uses whole-cell suspensions. Nucleoid samples are isolated after an interval sufficient to complete early strand-break repair. At this time point, the halo-comet

Table 5. Potential biosimetry cytogenetic assays.

Assay	Sample type	Post-sampling incubation (37°)	Processing	Persistence
Halo-comet	Blood	No	Short	Yes
Comet	Blood/tissue	No	Short	No
Apoptosis	Blood	No	Short	Yes
Dicentric	Lymphocyte	Yes (48 hr)	Long	Yes
PCC/Dicentric	Lymphocyte	Yes (30 min)	Short, complex	Yes

assay focuses on persistent DNA conformational effects, which can be used as a radiation biodosimetry assay system. The halo-comet assay has the potential to estimate partial-body exposure effects.

The halo-comet assay can also measure cell apoptosis, which can be considered a complementary or alternative biodosimetry measure. Most of the DNA in apoptotic comets moves from the comet's head into the tail. The apoptotic cell-tail moment is generally a factor of 15X to 30X greater than that exhibited by normal cells under standard assay conditions. The cytogenetic halo-comet assay allows detection of a higher fraction of apoptotic cells at an earlier time in comparison to flow cytometric methods.

The halo-comet scoring is the same as that performed for the normal comet assay. The principal halo-comet scoring measures include: 1) tail length which measures the distance of DNA migration from the nucleus, and 2) the tail moment which is the product of the tail length and the fraction of total DNA in the tail. The tail moment incorporates both size of migrating DNA (reflected in the comet tail length) and the number of fragments (represented by the intensity of DNA in the tail). Bivariate analysis using both DNA damage and DNA content is used to define subtle changes in response to various treatments in cells within the cell cycle.

Automated Halo-Comet Analysis System Design

The automated halo-comet cytogenetic system has been designed around existing hardware. The rapid automated cell-finding capability incorporated into the present design is based on the experience gained from developments and improvements of the automated metaphase finder, currently in service in the AFRRI Biodosimetry Laboratory (fig. 11). A cartridge-type slide container which can serve as a basis for slide preparation is
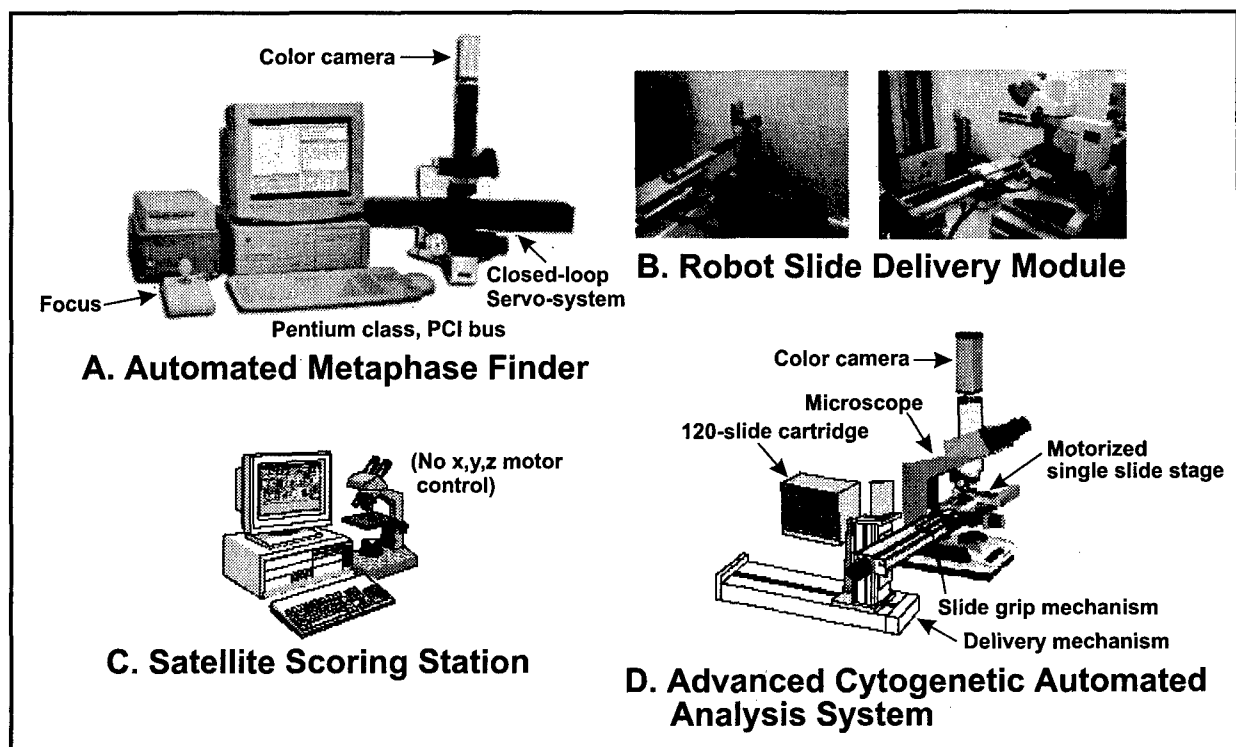
Fig. 11. Available hardware for cytogenetic assay automation. The automated halo-comet assay is based on modifications to the existing automated cytogenetic assay designed for Metaphase spread finding and analysis. The LAI Automated Metafind System (A) typifies the current state of cytogenetic assay automation. Important enabling extensions to this technology include an automated robotic slide delivery module (B) and stand-alone satellite scoring stations (C) that increase assay scoring throughput. An advanced system configured for use in the pharmaceutical industry is shown in panel (D). This system incorporates the large capacity robot slide delivery module required to accommodate high throughput requirements.

incorporated. Servo-controlled slide selection and delivery components are computer controlled. This capability is incorporated into the system that is intended for use in the field.

The LAI comet assay system employs a specially gated, cooled CCD camera that enhances the signal during image acquisition by integrating multiple acquisition frames. Comet images are automatically corrected for background and are analyzed as acquired. Non-target cells are automatically excluded from the analysis. An automated texture-based algorithm is used to define the discontinuous boundary of the comet tail.

Figure 12 illustrates the output of the LAI automated comet scoring system. Specific analysis parameters include: moment, moment arm, total-tail intensity, tail area, tail length, percent of tail DNA. Head and tail profiles are overlaid on each display. Group histograms of measured parameters are displayed and updated as the comets are acquired. Group statistics are available immediately upon completion of group acquisition. Histograms of group statistics are also continually displayed.

Multi-Channel Automated Halo-Comet Scoring System

The design guidelines adopted for the adaptation of the current automated comet assay system to accommodate military forward-fielding requirements are: 1) incorporation of minimized micro-optics, 2) incorporation of electronic zoom, 3) parallel cell search and analysis, and 4) the provision of multiple cell analysis channels. These criteria were

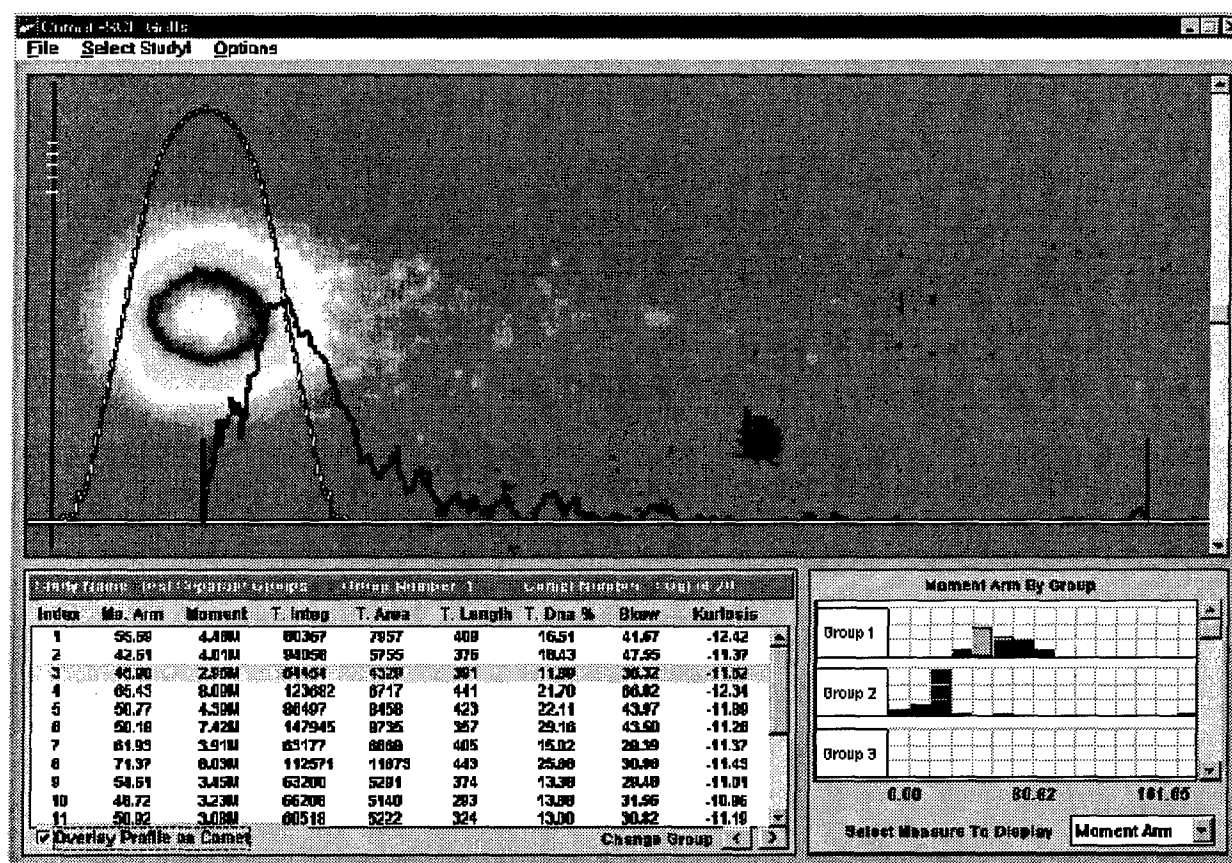**Fig. 12.** Current comet assay system at LAI. Shown here is the computer display of the LAI Automated Comet Assay program displaying a typical cell which has been automatically acquired and analyzed. The program searches and locates individual cells and automatically separates the comet head and tail. Quantitative measures include tail area, tail length and tail moment. Group statistics are accumulated as required.
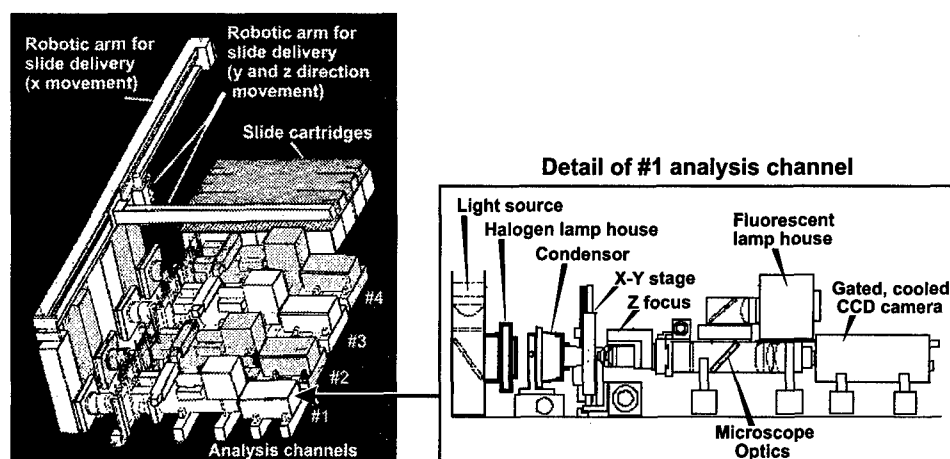
Detail of #1 analysis channel

Fig. 13. Halo comet field automated analysis system. This figure illustrates the design of a parallel multi-channel system proposed for the halo-comet assay. Individual slides are presented to the optical analysis channel by the robot slide delivery module. The fluorescent cell images are acquired with a special gated, cooled CCD camera acquisition module that provides a 12-bit image capture capability that is necessary to span the dynamic range of the cell images.

incorporated into the design of the multi-channel automated halo-comet system (fig. 13).

The special features of this system include: 1) automated cell finding, 2) a new technique for image acquisition based on low-light level range extension to provide dynamic range extension, 3) a digital method to produce a composite image from a series of closely spaced images neighboring the plane of best focus, and 4) parallel cell finding and analysis.

Throughput Estimation for the Automated Halo-Comet Assay

Based on the parallel cell finding/analysis strategy and multiple channel techniques, the following throughput estimates were developed. Time estimates for the multiplane image formation and scoring were directly extrapolated from existing program performance at AFRRI and LAI.

System throughput (T_p) is a direct function of the cells scored per patient (C_p), the number of patients (P) and the time to score a single cell (t_s). This total-time requirement is inversely proportional to the number of parallel-analysis channels (C). This relationship is defined in the following equation:

$$T_p = \frac{C_p \times P \times t_s}{3600C}$$

The time to score a single cell (t_s) was experimentally determined at between 4 and 10 seconds. This assumes that slide delivery and scoring are performed in a parallel fashion.

Figure 14 presents the throughput in patients scored per hour as a function of the number of channels for 500 patients at three levels of sample size (50, 100,

C_p = cells/patient; P = 500; T = 5 sec

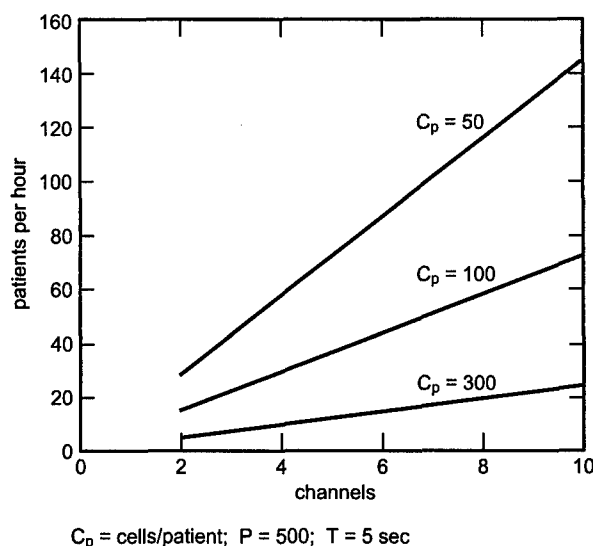**Fig. 14.** The effect of multiple scoring channels on the throughput of the cytogenetic halo-comet assay. This graph shows the performance of a multi-channel automated halo-comet assay system. The curves are parametric to the number of cells scored per patient and span the range required by triage and dose assessment. High patient throughput is achieved by increasing the number of analysis channels.

300 cells/patient). These sample sizes span the range of requirements for both the triage and the dose assessment modes of operation. In operational settings, a mixed strategy combining the triage time for the total-patient population with the scoring time for a fraction of the patient population would probably be used.

Table 6 compares the two levels (triage and dose assessment) for three patient-population levels (50, 100, and 500 patients) as a function of the scoring platform size as measured by the number of parallel scoring channels. This table shows that a multiple channel platform with parallel processes can automatically score large patient populations in short analysis times. The system works without human intervention. Image data can also be saved for review on a low-cost computer analysis station.

painted micronucleus assay. All of these assays have relevance to radiation bioassay with the exception of the SCE, which is related to chemical exposure.

In addition to their use in radiation biodosimetry, these assays and their conversion to an automated technique, can play important roles in general medical and clinical assays. Their adoption to general hematologic requirements is relatively straight forward and has been demonstrated in several semi-automated versions.

An added advantage of the systems approach taken here involving proven techniques of microscopic cytogenetics is to provide the capability to do multiple assays on the same hardware. At the same time, the hardware complement used would be identical for both forward-field and rear-support facilities.

Conclusions

The proceeding analytical study was performed to investigate the feasibility of fielding an echelon II or echelon III halo-comet cytogenetic scoring system. Under the assumptions detailed in the report, and based on time to score comets and existing hardware capabilities, we conclude that halo-comet and apoptosis assays are viable candidates for a single-use or multiple-use assay platform. Our previous research efforts have shown that a similar conclusion can be drawn for cytogenetic assays employing color-pigmented dicentric, sister-chromated exchange (SCE), and centromere-

Acknowledgments

Table 6. The effect of patient population size and C_P on total time requirement.

Number of parallel channels	Time to Score (hours)					
	50 Patients		100 Patients		500 Patients	
	Triage	Dose	Triage	Dose	Triage	Dose
2	1.7	10.4	3.5	20.8	17.4	104.2
4	0.9	5.2	1.7	10.4	8.7	52.1
6	0.6	3.5	1.2	6.9	5.8	34.7
8	0.4	2.6	0.9	5.2	4.3	26.0
10	0.3	2.1	0.7	4.2	3.5	20.8

Triage = 50 cells/patient   Dose Assessment = 300 cells/patient t_s= 5 sec

Potential Use of *In Vivo* Electron Paramagnetic Resonance, Electron Spin Resonance (EPR, ESR) for *In Vivo* Dosimetry Under Field Conditions

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Introduction

The recent development of *in vivo* electron paramagnetic resonance (EPR) techniques, combined with the fact that ionizing radiation generates relatively stable unpaired electron species in hard tissues, makes it feasible to carry out *in vivo* dosimetry with EPR. Such measurements are possible under conditions that will likely be present at forward deployed medical stations.

The occurrence of long-lived radiation induced EPR signals was demonstrated a number of years ago [1]. In hard and/or dry tissues such as bone or teeth these resonances were shown to provide accurate dosimetry at doses as low as 80 rads, even with the equipment available in 1968. It was suggested then that with improvements in techniques and/or the use of other materials, EPR could be used for dosimetry of unplanned exposures from even lower doses of ionizing radiation [2]. Subsequently, EPR technology has developed considerably. In the last few years *in vivo* EPR has been developed and applied successfully to a number of biomedical problems, leading to the likelihood of increased use in human subjects [3].

In addition to direct measurements made *in vivo*, a number of other approaches using EPR dosimetry could provide sensitive and relatively easy measurements of radiation exposure in the field. These include the use of samples which can be removed from subjects (e.g., nail clippings, hair and articles of clothing) and analyzed in highly sensitive instruments. Clothing could be made more sensitive and specific by developing functional uniform components, such as the development of buttons from material with a sensitive and reproducible EPR response to ionizing radiation.

The following sections describe in greater detail EPR's dosimetric potential to obtain useful data under field conditions. It is assumed that under these conditions the most pertinent information would help commanders and medical personnel differentiate between exposures which are 1) insignificant, or 2) significant but unlikely to cause acute symptoms, or 3) likely to result in significant early symptomatology. While it is likely that EPR dosimetry systems could be developed to provide data that are much more precise than this tripartite division for triage, the descriptions in this paper are aimed at achieving quickly the information needed for decision making in the field. It is further assumed that under battlefield conditions, the initial premise will be that significant exposures occur more or less uniformly throughout the body; therefore, dosimetry from any one point or from pooled samples from several sites on the body are sufficient to provide the needed information.

Sites (Samples) To Be Used

On the basis of existing information and results, the most effective site for EPR dosimetric measurements is the teeth. It has already been demonstrated that this tissue has the most sensitive dose-response relationship [2]. The radiation-induced EPR signal in teeth has distinctive characteristics based on its shape and power saturation. The use of teeth also has the advantage of providing a site where good geometry with a sensitive resonator can readily be achieved for entirely non-invasive measurements *in vivo*. It should be possible to place a specially designed resonator around the teeth *in vivo* so that maximum sensitivity is obtained by having the "sample" in the region of highest sensitivity for the resonator; alternatively, a surface type resonator could be used. Because of the relatively low dielectric loss in teeth, it may be possible to use higher frequencies than are ordinarily used for *in vivo* EPR. Although only preliminary results have been reported to date, it seems possible that usable sensitivity could be obtained with the use of fingernail and toenail cuttings. The advantage of these specimens includes the capability to pool samples so that a relatively large mass could be obtained, which could then be measured with a high sensitivity EPR

spectrometer (e.g., 9 GHz, X-band). Additionally, it would be feasible to have an irradiation source available in the field for calibration purposes.

The use of hair has considerations similar to those for toenails and fingernails. In some ways it would be even easier to obtain hair samples in relatively large amounts. The potential disadvantages are the presence of a background signal due to melanin [4] and the potential for loss of signal if the hair is washed [2]. However, it should be feasible to differentiate the EPR signal from melanin on the basis of its line shape and microwave power saturation characteristics. These spectroscopic properties could then be used to implement an automatic method to calculate the signal from melanin and subtract it from the total signal; this would allow the radiation-induced signal from hair to be evaluated accurately.

The use of articles of clothing has advantages similar to those for hair and nails; i.e., these samples could be studied using high sensitivity EPR spectrometers. Perhaps the most attractive aspect involves designing functional articles of clothing such as buttons to serve as emergency dosimeters for assay by EPR. Virtually all solid materials have detectable radiation-induced EPR signals under appropriate conditions. Sensitive and effective dosimetry systems have already been developed based on responses by the amino acid alanine. These systems are now capable of measuring doses well below the requirements for decision-making under field conditions. Although alanine based dosimeters are now widely used, it is clear from a spectroscopic point of view that alanine, because of its complex and wide-line shape, is far from an ideal EPR dosimeter. However, it should be relatively straightforward to find plastic materials with better suited radiation-induced stable EPR lines. Such materials could be incorporated into uniform parts, such as buttons.

Instrumental Aspects

Direct *in vivo* EPR usually requires the use of EPR instruments which operate at lower frequencies, such as 1 Ghz [3]. This is because large amounts of materials, especially water, which non-resonantly absorb the frequencies used for conventional EPR spectroscopy (9 GHz), are present in tissues. While *in vivo* EPR spectroscopy has been developing rapidly and is being used increasingly for many studies, it does have significantly less sensitivity than EPR spectroscopy which uses conventional higher frequencies. Teeth, however, are an exception to the general rule that tissues are composed of 70% to 90% water; therefore, the sensitivity of EPR for studies of teeth should be higher than for most tissues. Precise data on these differences are not available presently but could be readily obtained in days or weeks.

Such studies would then facilitate the decision as to the appropriate instrumental conditions to use for EPR dosimetry under field conditions. Key variables to consider are the amount of incident microwave power that can be used effectively for studies within the mouth, and the resulting signal to noise ratio. These factors, in turn, would be affected by the choice of frequency and the type of resonant structure that is employed. The frequency and strength of the modulation would be another important factor to optimize. In general, one would aim to use the highest practical frequency to make measurements with teeth under *in vivo* conditions. If it is determined that a frequency higher than 1 GHz would be applicable for study of teeth *in vivo*, the principles that already have been implemented for the construction of *in vivo* EPR instruments at 1 GHz could be utilized.

The implementation of a system to use *in vivo* EPR to make measurements of radiation exposure under field conditions seems relatively straightforward. Although the following is based on our experience in developing *in vivo* EPR at 1.2 GHz, the approach is similar for most applicable frequencies. The engineering of a system that could be operated in the field for this special use should present no particular difficulties. The construction of the microwave bridge and control system can be designed for rugged use and portability. The controls and output can be configured, using a portable computer, so that an operator with minimal technical background can obtain an unambiguous result in the form of a number that indicates the extent of radiation exposure. The power requirements for this part of the spectrometer are modest.

The strength and configuration of the magnetic field would depend on the choice of spectrometer operating frequency. If a lower frequency spectrometer is chosen, then either a large magnet in which the subject would be positioned between the magnetic poles could be used; or, a structure could be developed in which a small magnetic field of appropriate strength is generated at the site where the measurements will be made. The latter configuration would be the likely method of choice if a higher frequency EPR spectrometer will be used; this would keep the size of the instrument suitable for field use. We recently carried out a detailed feasibility study for a fully portable instrument at 1 GHz, using a magnet system into which the subject was placed. We tested some of the critical components and did not find any crucial limitations.

The choice of resonator is important, but could be made from among those which have been developed and are already in use. The previously described coupled circuit with a loop is the most likely resonator configuration [3]. Such a resonator could be constructed to allow the loop to be placed around the tooth to be measured. This would provide the best geometric condition for achieving an optimum signal to noise ratio. Alternatively, a surface resonator [5], which could be shaped to fit the contours of teeth, could be used. There might be some advantages in using a dielectric resonator [6]. The use of a frequency of 1.1 GHz would be the simplest system to implement quickly. To maximize sensitivity, serious consideration should be given to systems that operate at higher frequencies.

A conventional EPR instrument could be used on samples that are removed from the subject (e.g., nail clippings, or articles of clothing). The sample would be placed at an optimum position within a fixed magnet system. Such an instrument also could be developed for dual use, to measure *in vitro* objects of clothing, etc., which might have even more readily detectable radiation-induced EPR signals [2].

Any of the instruments described above could be designed to be operated by minimally trained personnel and could be used for screening large numbers of people. At least initially under these circumstances the results would be semi-quantitative, and would be sufficient to provide firm guidance for decision making in the field. Such an instrument could be designed to indicate a radiation dose within the precision needed for the purpose.

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EPR-Based Dosimetry and Its Present Suitability for Field Usage

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The recent development of a small and relatively portable EPR analyzer with nearly the sensitivity of a research instrument opens the possibility of bringing EPR measurements to the field [1].

Electron Paramagnetic Resonance has long been accepted as an accurate and reliable method for the qualitative and quantitative determination of the effects of ionizing radiation. Work to date has been performed on research instruments which, due to their size and weight, are confined to a laboratory environment. The EMS 104 EPR Analyzer (Fig.15.) is small by comparison and requires neither cooling water nor three-phase power, making it suitable for field use. Development of a practical computerized control system also provides quantitative measurements with minimal operator training. Appropriate software has been developed for assays using several distinctly different dosimetry systems.

Electron Paramagnetic Resonance (EPR)

Unpaired electrons are created by ionizing radiation; and their presence can be used to measure the radiation dose administered to a suitable sample.

Electron Paramagnetic Resonance is a form of spectroscopy that observes unpaired electrons in various substances by stimulating transitions between normally degenerate Zeeman energy levels separated by the application of an external magnetic field. The EPR Spectrometer consists of a magnet system capable of producing the required field, typically .35T, and suitable field sweep capability to display the spectral region of interest. The sample is placed in a microwave rf field whose frequency corresponds to the separation in Zeeman levels caused by the external field, typically 10 GHz. The absorption of energy by the sample is then plotted as a function of field. Computerization of the instrument allows automatic acquisition of the signal and computation of the applied dose. A block diagram of the EMS 104 is shown in Fig. 16.

Alanine

Crystalline alanine when subjected to ionizing radiation is converted predominantly to a stable paramagnetic species, CH_3-C*H-COOH. The 5-line EPR spectrum is well characterized and the amplitude of the central line is proportional to applied dose. Thus, the use of alanine pellets and films for dosimetry in radiation processing provides the ease and convenience of a long-lived, cumulative, re-readable dosimeter that can be attached to products or carried by personnel [2]. These dosimeters can be packaged and labeled for easy identification and read using automated equipment; doses are displayed directly in Gray by the instrument. Alanine
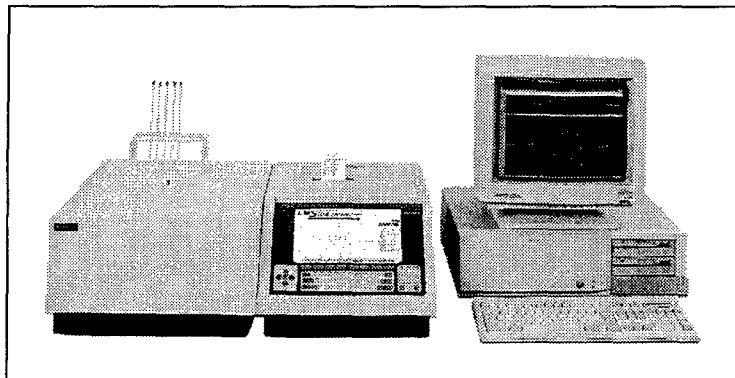

Fig. 15. The EMS 104 EPR Analyzer requires neither cooling water nor three-phase power—suitable for field use.
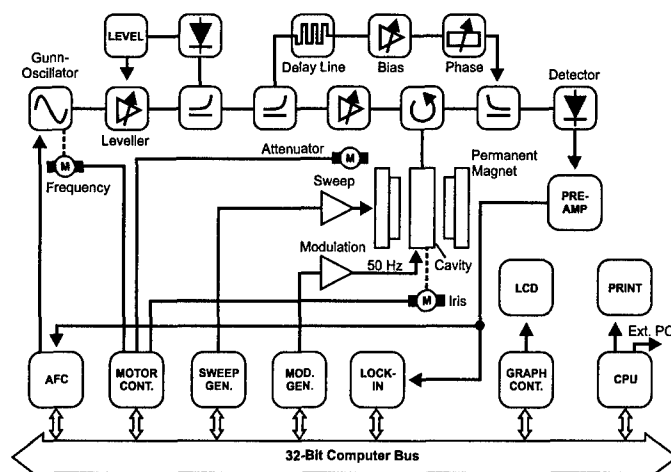
Fig. 16. A block diagram of the EMS 104.

is currently well established as a transfer standard dosimeter in the radiation processing community; and an ASTM standard (E 1607) exists detailing procedures and usage of alanine pellets.

The useful range of alanine pellets, typically 5mm diameter x 5mm height, is from ca. 1 Gy to 10^5 Gy using commercially available pellets. This range can be extended to lower dose levels by increasing the size of the sample and the measurement time; although measurements below 0.1 Gy are difficult. Alanine films are also commercially available. Being flexible, they may be incorporated in or attached to clothing or other sheet materials. However, with a lower mass, they have higher minimum detectable dose levels.

Alanine dosimeters have been shown to be independent of dose rate. They are suitable for beta, x-ray, and gamma radiation, and have a minimal temperature dependence and linear response up to tens of kilograys with excellent polynomial fits at higher doses. Readout for doses greater than a few gray can be performed in seconds with high confidence levels. A typical segment of the alanine-derived radical spectrum (the central line of a five-line spectrum) used for analysis is shown in Fig. 17; and a typical calibration curve is shown in Fig. 18.

Spin Label Assays

The development of spin-label assays for radiation dose using whole blood provides another possibility for field use of EPR. The preparation of a suitable sample requires only a few minutes; and the sample can be read in seconds. Spin labels do not have adverse problems associated with radioisotopes and can be stored indefinitely. Changes in receptor-binding properties of human red blood
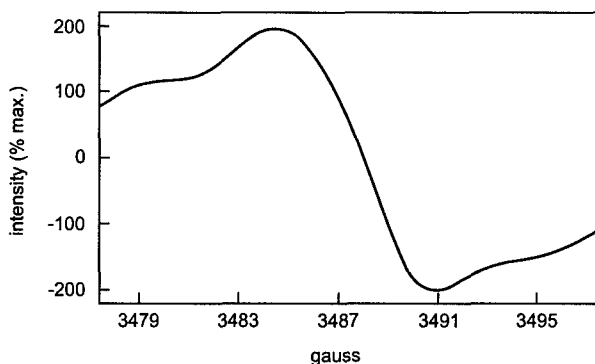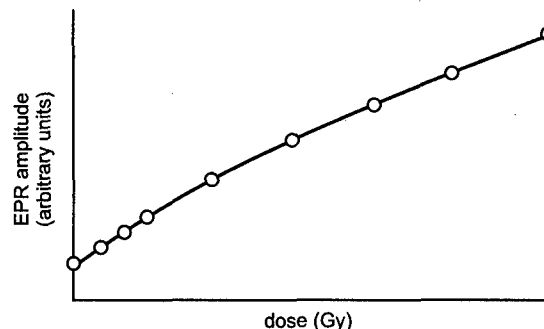

Fig. 17. A typical segment of the alanine-derived radical spectrum (the central line of a five-line spectrum) used for analysis.

Fig. 18. Typical calibration curve for alanine dosimeters.

cells have been studied at the membrane level using spin labeled insulin. Preliminary studies made at the Armed Forces Radiobiology Research Institute (AFRRI) have shown a distinct and measurable difference in the number of binding sites measured between the unirradiated and irradiated cells. Further work is needed to better characterize the effect and determine dose vs. binding curves. However, this method shows promise both as a means of determining levels of irradiation applied to blood used for transfusion and blood drawn from accident victims.

Tooth and Bone

The use of bone samples is also well established for determining exposure to ionizing radiation and is an established method in Europe where irradiation of foodstuffs is prohibited in certain markets. The major problem with bone measurements is the uncertainty in determining the background, as well as the wide variation in sample density [3]. EPR measurements to determine dose in bone usually require the exposure of the sample to additional radiation to establish a dose-response curve. Back-extrapolation is then used to determine the initial dose. This is easily accomplished in the laboratory but would be unsuitable for a field measurement.

Since tooth enamel contains 95–98% hydroxyapatite it is more suitable for field use in dosimetry than bone; as enamel is more uniform and contains fewer impurities. Considerable research has been done in determining dose using EPR measurements on extracted teeth. Ikeya and others have done extensive work on the teeth of A-bomb survivors and developed suitable dosimetry methods [4].

Some developmental work has been done on EPR measurements of unextracted incisors using portable magnets and a surface probe. The higher sensitivity of the EMS 104 and its modular nature make it adaptable to incorporating such a probe as an extension to the existing instrument. The use of the EMS 104 electronics in combination with an MRI magnet's fringe field would considerably lessen the difficulties of bringing this technique forward, as in the case of screening accident victims. The growing availability of MRI systems in most populated areas makes this a reasonable consideration. The experimental problem is reduced from having to produce the main external field to producing only the field sweep and modulation; a task that is made considerably less difficult by utilizing existing EMS 104 electronics.

In addition, work on shell buttons has shown significant sensitivity in the concentration of CO_2 to ionizing radiation in the 0–10 Gy range. Spectra similar to those obtained from fossil shells are obtained; and results consistent with other dosimeters are found. Distribution of buttons of suitable inorganic or polymer materials would be innocuous. These could be attached to clothing and read following exposure with few problems. Determining background dose would cease to be a problem as this could be determined for an entire batch beforehand in the laboratory.

Bruker Instruments, Inc. has developed several instruments for military field use, including the MM-1, the first mil-spec fully mobile chemical warfare agent mass spectrometer for military warfare agent detection, and is currently testing, under a current government contract, a Bruker developed mass spectrometer with bio-agent detection for use on a battlefield with chemical contaminants. As a result, the extension of these efforts to produce a rugged, portable EPR spectrometer is well within the capability and scope of the company's manufacturing facilities in the United States and Europe. It remains to be seen if sufficient demand for such an instrument exists to prompt further development.

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Appendix E

List of Attendees
WORKSHOP ON TRIAGE OF IRRADIATED PERSONNEL
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13. ABSTRACT (Maximum 200 words)

AFRRI was tasked by the Army Office of the Surgeon General to answer operational questions concerning three issues related to the triage of irradiated personnel deployed forward. These issues, and the workshop participants' consensus responses, are:

1) **Effects of radiation injuries on exposed personnel, assuming Level I and Level II medical care and facilities.** Individuals receiving over 1.5 Gy should be evacuated; those receiving less than this amount may return to duty. Even at this level, 30% of exposed personnel may be too ill to return to duty. Those who recover will experience varying degrees of persistent fatigue and weakness. Dose assessment at this level is best served by physical dosimetry.

2) **Describe these effects in previously exposed personnel.** Physical dosimetry is required for personnel who are at risk of a second exposure; no animal model completely predicts the effects of either protracted or multiple radiation exposure in humans. Fatigue and weakness in multiply exposed personnel will be cumulative.

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